Universidad de Alcalá
Departamento de Ciencias de la Vida. Unidad docente de Ecología

Physicochemical and macroinvertebrate community trends in manmade ponds constructed in reclaimed opencast coal mines

PhD THESIS

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Tendencias en la fisicoquímica y la comunidad de macroinvertebrados en balsas de nueva creación en minas de carbón a cielo abierto recuperadas

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Alcalá de Henares, a 27 de septiembre de 2013,

Dr. Gonzalo Pérez Suárez

A mis padres,

"Ensuring that goals are both explicit enough
to be meaningful and realistic enough to be achievable
is a key to the development of successful projects"
(Hobbs, 2003)

CONTENT

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ABSTRACT

ENGLISH VERSION

The increase in the number of artificial ponds during the last decades has not been accompanied by scientific studies about the ecological functioning of these ecosystems over time. Indeed, knowledge about long-term evolving of artificial manmade ponds is still scarce in physicochemical and biological characteristics. Ponds have been constructed with different purposes, from compensating loss of natural wetlands to treating wastewater. We have focused our attention in ponds constructed in reclaimed opencast coal mines for the control of runoff. The main objective of these manmade ponds was to avoid the pollution of natural ecosystem downstream reclaimed mines. Additionally, these manmade ponds may provide interesting ecological functions in a context of global lost of natural lentic ecosystems. But, the kind of ecological functions and services that the manmade ponds may provide are going to be determined by their characteristics. On the other hand, manmade ponds represent an excellent scenario for analyzing ecological processes as the primary succession because they are created on places where the aquatic community has not previously existed and the pond age is usually known. The information about these subjects is really scarce so, in this PhD dissertation we aspire to improve the knowledge about the characteristics of the aquatic ecosystem constructed in reclaimed opencast coal mines over time and the primary succession in manmade ponds. To that end, 19 permanent manmade ponds constructed in reclaimed opencast coal mines in the Northeast of Spain (Teruel province) covering a range of 22 years old were sampled in spring and summer of 2009. In order to get a whole consideration of the changes of the aquatic ecosystem over the time, we studied the macroinvertebrate community, the dominant macrophytes and the water, sediment and landscape characteristics of the manmade ponds.

First we explored the environmental conditions of the manmade ponds to evaluate their metal pollution over the time. We focus this study in the metal pollution of aquatic ecosystems because it is an important challenge of mining industry and manmade ponds are considered good sinks for metals. We assessed the three main compartments involved in the process of heavy metal removal: water, sediment and aquatic macrophytes. We only use the data of 17 manmade ponds because in two ponds *Typha* sp. (the dominant macrophyte) was absent. In addition we sampled a pit-lake formed in an un-reclaimed

opencast coal mine in order to compare water bodies in reclaimed and un-reclaimed areas. Ten heavy metals (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) were analyzed in water, sediment and *Typha* sp. tissues. In addition, other physicochemical variables such as pH, conductivity, alkalinity and sulfur were analyzed in water and sediment. The ponds constructed in reclaimed coal mines showed neutral pH but their chemical composition revealed a clear coal mining influence along the chronosequence: high and increasing concentrations of SO_4^{2-} and stable but toxic concentrations of dissolved Al, Cd, Cu and Ni in water; and high and increasing concentrations of total Fe and Al and toxic but constant concentration of As and Ni in sediment. The presence of toxic concentrations of heavy metals over the time compromises the development of the aquatic community. Moreover, the metal concentration in the macrophytes were higher than medium concentration of metals in plants and that metal concentration of *Typha* sp. in un-polluted sites. Despite the metal pollution, the manmade ponds showed better characteristics than the pit-lake with acidic pH and higher and toxic concentrations of dissolved heavy metals. Therefore, our findings highlight the importance of reclaiming opencast coal mines in the control of metal pollution. But also, suggested the possibility of chronic metal pollution in the reclaimed coal mines since metals remain as an environmental problem at least 19 years after the mining reclamations.

After the environmental evaluation of manmade ponds, we focused our analysis on the primary succession of the macroinvertebrate community. First, we questioned how the taxonomic biodiversity changes during primary succession in relation with the age and the environmental factors of the manmade ponds. Then, we considered the taxonomic and functional changes of macroinvertebrate community over time. We considered four biodiversity metrics to study the biodiversity of the macroinvertebrate community over time: rarefied richness, average taxonomic distinctness (AvTD), variation taxonomic distinctness (VarTD) and rarity (IFO). To know how the community biodiversity change over time the 19 manmade ponds were grouped in four age categories (Pond Age Categories, hereafter PAC): PAC1, 1–5 years (5 ponds); PAC2, 6–10 years (5 ponds); PAC3, 11–15 years (4 ponds); and PAC4, 16-22 years (5 ponds). We considered water, sediment and landscape characteristics in addition to pond age to evaluate what kind of factors explained better the biodiversity changes. This study showed an increase of the macroinvertebrate complexity with the PAC. The oldest ponds (PAC4) showed more distant taxonomic relationships (AvTD) a greater unevenness in their taxonomic tree (VarTD) and higher number of rare taxa (IFO) than youngest ponds (PAC1). But taxonomic richness did not change over time. These results contribute to support that no single metric alone is a suitable surrogate for representing overall community biodiversity, especially taxonomic richness. Despite the increase in the complexity of the macroinvertebrate assemblage over time, the variation partitioning showed low contribution of pond age to the explanation of the biodiversity of the manmade ponds. Rather, environmental data sets explained a greater proportion of the macroinvertebrate biodiversity. Moreover we have detected that the biodiversity in the manmade ponds constructed in the reclaimed coal mines was lower than in other constructed ponds of similar ages. Therefore, the macroinvertebrate evolving could be limited by the environmental characteristics of the manmade ponds among which the metal pollution may stand out.

Because the isolated character of the manmade ponds, we expected that the primary succession were affected by the dispersal abilities of the organisms. Thus, we divided the community in two groups, poorly and easily dispersing organisms, where easily dispersal included the better aerial dispersers. Similar to the biodiversity study, we used the 19 manmade ponds grouped in PAC to study the taxonomic and functional evolving of the macroinvertebrate community and we calculated analysis of similarity and correlations. Our study revealed that the macroinvertebrate community has different responses during primary succession depending on their dispersal abilities. We did not found taxonomic differences among PAC in either poorly or easily dispersing organisms. At functional level, several changes were detected among youngest and oldest ponds. The changes in poorly dispersing organisms lied in the affinity increase for larger body sizes, longer life cycles and higher trophic positions (i.e., predators) along succession (i.e. PACs). So, according to our expectations, poorly dispersing organisms showed a slightly shift from *r* to *K* strategies. Changes detected in the easily dispersing organisms were less predictable. The long-term study of the macroinvertebrate community additionally allows investigate the forces that could be driving the primary succession. Our results highlighted the effect of historical contingent forces (biological interactions and stochasticity) in the primary succession. Poorly dispersing organisms diverged in taxonomic composition while their functional characteristics maintained high similarity values over time. Moreover we found similar environmental characteristics among PAC2 to 4 so, because the community was changing during this period, historical contingent forces revealed also important. The similarity of poorly dispersing organisms was higher than the similarity of easily dispersing organisms suggesting that organisms with reduced dispersion could be more influenced by the environmental conditions. Finally, in accordance to the biodiversity study, the lack of taxonomic differences and the weak functional differences observed in the community among PAC suggested that isolated ponds in reclaimed opencast coal mines have limiting environmental characteristics for the primary succession of macroinvertebrates.

The manmade ponds may provide ecological functions and socio-economic services complementary to the main objective of retain the mining runoff. These functions and services are particularly attractive in regions where natural wetlands and ponds are virtually absent as was the case of this study site. Nevertheless, our study reveals that manmade ponds constructed in reclaimed mines may be metal polluted. This fact is going to determine the evolving and the social use of the manmade ponds. The metal pollution and the low biodiversity should be considered in mine reclamation and restoration plans in order to reduce metal concentrations under toxic levels and improve the biodiversity of post-mining landscapes. In addition, research on primary succession and other ecological processes in lentic ecosystems may be also integrated in mine reclamation and restoration plans.

Key words: primary succession, macroinvertebrate community, biodiversity, richness, taxonomic distinctness, rarity, dispersal ability, taxonomic approach, functional approach, long-term, manmade pond, opencast coal mining, reclamation, heavy metals.

SPANISH VERSION

En las últimas décadas el número de balsas construidas por el hombre ha crecido considerablemente. Sin embargo, el aumento en el número de balsas artificiales no ha sido acompañado por estudios científicos de carácter ecológico acerca de sus características y su funcionamiento a lo largo del tiempo. De hecho, el conocimiento que se tiene sobre la evolución a largo plazo en balsas artificiales es todavía escaso desde los puntos de vista fisicoquímico y biológico. Las balsas artificiales se construyen con objetivos muy distintos, desde compensar la pérdida de humedales naturales hasta el tratamiento de aguas residuales. En este caso, la investigación se ha centrado en balsas construidas para controlar la escorrentía que se produce en minas de carbón a cielo abierto que han sido recuperadas. El principal objetivo de estas balsas es evitar que la escorrentía generada en las minas salga fuera de las zonas mineras recuperadas y que afecte a los ecosistemas naturales. Además, estas balsas podrían proporcionar funciones ambientales muy interesantes en un contexto de pérdida y degradación generalizada de los ecosistemas acuáticos. Sin embargo, el tipo de funciones y servicios que puedan cumplir estarán condicionados por sus características y el nivel de contaminación que soporten. Por otro lado, este tipo de balsas ofrecen un excelente escenario para analizar procesos ecológicos como la sucesión primaria ya que están creadas en lugares donde no había una comunidad acuática previa y se conoce su edad aproximada. La información existente acerca de estos aspectos es realmente escasa, por lo que los objetivos de este estudio fueron: conocer las características de las balsas creadas en minas de carbón a cielo abierto recuperadas e indagar acerca del proceso de sucesión primaria en sistemas artificiales. Para estos fines, se muestrearon 19 balsas artificiales construidas en minas recuperadas de carbón a cielo abierto en la provincia de Teruel, al este de España. Estas balsas cubrían un rango de edad de aproximadamente 22 años desde su creación y en ellas se tomaron muestras de macroinvertebrados, macrófitos, agua y sedimento y se estudiaron las características del paisaje. Los muestreos fueron realizados en primavera y verano de 2009.

En primer lugar, se exploraron las condiciones ambientales de las balsas artificiales en función de su edad. El estudio se centró en examinar el contenido en metales pesados ya que la contaminación por metales es uno de los grandes impactos ambientales producidos

por la minería en todo el mundo, incluso en zonas que han sido recuperadas. Además, las balsas son consideradas buenos acumuladores de metales. Se analizaron el agua, el sedimento y los macrófitos acuáticos debido a que son los principales compartimentos involucrados en el proceso de retención de metales. Se utilizaron datos de 17 de las 19 balsas ya que en dos de ellas no crecía *Typha* sp. que es el macrófito dominante. Además, se muestreó un pit-lake ubicado en una mina abandonada dentro de la misma zona de estudio. Los pit-lakes son lagos que se forman en el hueco final de extracción de una mina a cielo abierto una vez que la extracción minera ha finalizado. El pit-lake nos permitió comparar ecosistemas acuáticos en zonas recuperadas con aquellos que se formarían si las minas hubieran sido abandonadas. Diez metales pesados (Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) fueron analizados en agua, sedimento y en los tejidos de *Typha* sp. Además, se analizaron variables como el pH, conductividad, alcalinidad y el contenido en azufre en el agua y el sedimento. Las balsas construidas para el control de la escorrentía mostraron un pH neutro, aunque su composición química reveló una clara influencia de la minería del carbón a lo largo del tiempo. En el agua, se detectaron elevadas concentraciones de SO_4^{2-} que aumentaron con la edad de la balsa; además, se detectaron concentraciones tóxicas pero estables de Al, Cd, Cu y Ni. En el sedimento, se encontraron concentraciones elevadas de Fe total y Al total aumentando a lo largo del tiempo y concentraciones toxicas pero estables de As total y Ni total. La presencia de concentraciones toxicas de estos metales en las balsas compromete el desarrollo de la comunidad de organismos acuáticos. Además, el efecto de la minería también se detectó en los macrófitos ya que la concentración de metales en *Typha* sp. fue mayor que la concentración media de metales en las plantas y que la concentración de metales en *Typha* sp. en zonas no contaminadas. El pit-lake se caracterizó por tener pH ácido tanto en el agua como en el sedimento y concentraciones toxicas de metales superiores a las de las balsas. Por lo tanto, aunque exista contaminación por metales en las balsas artificiales, los resultados resaltan la importancia de la recuperación de zonas mineras del carbón para el control de la contaminación por metales y la obtención de ecosistemas funcionales. Pero además, sugieren la posibilidad de que la contaminación por metales en las balsas artificiales sea crónica ya que las concentraciones toxicas para los organismos permanecieron estables en un rango de 19 años después de la recuperación de las minas.

Después de analizar las características ambientales y el nivel de metales en las balsas artificiales, se analizó la sucesión primaria de la comunidad de macroinvertebrados. Primero, se exploró cómo la biodiversidad taxonómica cambia a lo largo de la sucesión primaria y los factores que influyen en esos cambios. Después se analizaron los cambios taxonómicos y funcionales de la comunidad a largo del tiempo. En el estudio de la biodiversidad de la comunidad de macroinvertebrados a lo largo del tiempo se tuvieron en cuenta cuatro medidas de biodiversidad complementarias entre sí: riqueza rarificada, distinción taxonómica media (AvTD), variación en la distinción taxonómica (VarTD) y rareza (IFO). Para conocer cómo cambia la biodiversidad de la comunidad a lo largo del tiempo las 19 balsas se agruparon en cuatro categorías de edad (PAC): PAC1, 1-5 años (5 balsas); PAC2 6-10 años (5 balsas); PAC3 11-15 años (4 balsas); PAC4 16-22 años (5 balsas). Además de la PAC, se consideraron las características del agua, sedimento y paisaje para estudiar los factores que influyen en la biodiversidad de las balsas. Este estudio mostró un aumento de la complejidad de la comunidad de macroinvertebrados con la edad. Las balsas más antiguas (PAC4) mostraron una relación taxonómica más distante (aumento de AvTD), una mayor desigualdad en el árbol taxonómico (aumento de VarTD), y un mayor número de taxones raros (aumento de IFO) que las balsas más jóvenes (PAC1). Sin embargo, la riqueza de taxones se mantuvo constante a lo largo del tiempo. Este resultado apoya la idea de que utilizar un solo índice de biodiversidad no es adecuado para representar toda la biodiversidad de la comunidad y estudiar sus cambios a lo largo del tiempo; sobre todo si éste índice es la riqueza taxonómica. A pesar del aumento de la complejidad de la comunidad de macroinvertebrados con el tiempo, el análisis de partición de la varianza mostró una baja contribución de la PAC en la explicación de la biodiversidad de las balsas. Fueron los datos ambientales los que explicaron una mayor parte de la biodiversidad. Además, se encontró que la biodiversidad de las balsas fue menor que la biodiversidad encontrada en otras balsas artificiales de edad similar. Por tanto, la evolución de la comunidad de macroinvertebrados podría estar limitada por las condiciones ambientales de las minas de carbón a pesar de haber sido recuperadas. Entre los factores limitantes podría destacar la contaminación por metales.

La mayoría de las balsas artificiales en nuestra área de estudio se encuentran desconectadas de ríos o arroyos. Por tanto, la sucesión primaria podría estar fuertemente condicionada por la capacidad de dispersión de cada organismo. Así, en el estudio de los cambios taxonómicos y funcionales a lo largo del tiempo, la comunidad de macroinvertebrados se dividió en dos categorías de dispersión: malos y buenos dispersores. En los buenos dispersores se incluyeron a los organismos que presentan mejor dispersión aérea. Al igual que en el estudio sobre la biodiversidad, en este caso se utilizaron los datos de las 19 balsas agrupadas en PAC. Este estudio reveló que la comunidad de macroinvertebrados tiene diferentes respuestas durante la sucesión primaria dependiendo de sus habilidades de dispersión. No se encontraron diferencias taxonómicas entre PAC ni en los malos ni en los buenos dispersores. A nivel funcional, se detectaron pequeños cambios entre las balsas más jóvenes y las más antiguas. Los malos dispersores aumentaron su afinidad por grandes tamaños, ciclos de vida más largos y posiciones tróficas más altas (predadores) a lo largo de la sucesión. Por tanto, estos resultados sugieren un cambio de estrategia para los malos dispersores desde la *r* hacia la *K*. Los cambios detectados entre las PAC para los buenos dispersores fueron menos estructurados. Además, el estudio combinado de las características taxonómicas y funcionales de la comunidad a lo largo del tiempo nos permitió investigar sobre el tipo de fuerzas que podrían estar dirigiendo la sucesión primaria. Nuestros resultados mostraron el efecto de las fuerzas contingentes históricas (interacciones biológicas y estocasticidad) en la sucesión primaria. Mientras que la similaridad funcional de los malos dispersores se mantuvo con valores de similaridad muy elevados durante las cuatro PAC, la comunidad se diferenció taxonómicamente con el tiempo. Además, en las PAC 2, 3 y 4 se encontraron características ambientales muy similares sugiriendo que las fuerzas contingentes históricas podrían ser las principales responsables de los cambios detectados en la comunidad durante ese periodo de tiempo. Por otro lado, la comparación de la similaridad funcional entre malos y buenos dispersores sugiere que los malos dispersores podrían verse más afectados por las condiciones ambientales que los buenos dispersores. Finalmente, las débiles diferencias encontradas entre las distintas clases de edad sugieren, de acuerdo con el estudio de biodiversidad, que balsas aisladas construidas en minas de carbón recuperadas pueden ser un ambiente limitante en el proceso de sucesión primaria.

Las balsas construidas en minas de carbón a cielo abierto con el objetivo de retener la escorrentía, además pueden proporcionar funciones ecológicas y otros servicios para la sociedad. Este tipo de funciones y servicios son especialmente interesantes en regiones como la estudiada donde humedales y balsas naturales son realmente escasos. Sin embargo, como se ha identificado en este caso, estas balsas pueden estar contaminadas por metales, lo cual va a condicionar su evolución y el tipo de actividades que se puedan realizar en ellas. La contaminación por metales y los bajos valores de biodiversidad deberían considerarse en los planes de recuperación mineros con el objetivo de reducir esta contaminación por debajo de niveles tóxicos y mejorar la biodiversidad de los paisajes mineros recuperados. Además, la investigación sobre la sucesión primaria y otros procesos ecológicos en ecosistemas acuáticos lénticos podrían integrarse dentro de los planes de recuperación.

Palabras clave: sucesión primaria, comunidad de macroinvertebrados, biodiversidad, riqueza, distinción taxonómica, rareza, aproximación taxonómica, aproximación funcional, balsas artificiales, minería de carbón a cielo abierto, recuperación, metales pesados.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Wetlands are ecosystems characterized by the presence of standing water for some period of time during the growing season, to have unique soil conditions and support vegetation adapted or tolerant to the wet conditions (Mitsch & Gosselink, 2000). Wetland covers a wide range of lentic aquatic ecosystems among which ponds are included. Particularly, ponds are natural or artificial water bodies characterized by small size, from1 m^2 to about 5 ha, and shallow deep, about less than 8 m (Biggs et al., 2005; Oertli et al., 2005; Céréghino et al., 2008a).

Because the small size of the ponds they have long been overlooked, although during the last decades the studies about these water bodies has been progressively increasing (Oertli et al., 2009; Downing, 2010). A wealth of studies have demonstrated the importance of ponds for their disproportionately rich biodiversity (Oertli et al., 2002; Williams et al., 2004; Biggs et al., 2005) and its role in geochemical cycles (Mitra et al., 2005; Zedler & Kercher, 2005; Downing et al., 2008). Additionally, from the socio-economic perspective, ponds provide important functions such as water supply, hydrological regulation, nutrient retention, fish production, wildlife protection, recreation and education roles (Hansson et al., 2005; Oertli et al., 2005).

Despite their intrinsic value, a great proportion of natural wetlands and ponds were lost worldwide during XX century (King, 1998; Gallego-Fernández et al., 1999; Williams et al., 1999; Zedler & Kercher, 2005). The loss percentage of wetlands varies from geographic areas. Usually the loss of wetlands were above the 50 % of wetlands has been lost, although in Europe the loss of wetlands reach the 90 % (Mitsch & Gosselink, 2000). In addition, most of the wetlands that remain have their permanence threatened and support increasing pressure due to eutrophication, acidification, contamination and invasion of exotic species as a result of human activities (Brönmark & Hansson, 2002; Hansson et al., 2005; Zedler & Kercher, 2005; Gascón et al., 2009).

Simultaneously to the loss of natural wetlands and ponds, the number of constructed water bodies is increasing. Following policies based on the concept of “no net loss” new water bodies have been constructed to try to reduce or compensate the loss of natural wetlands and ponds (Spieles et al., 2006; Ruhí, 2012). Moreover, water bodies have been created as result of extractive socio-economic activities (Barnes, 1983; Proctor & Grigg, 2006). Finally, wetlands and ponds have been usually constructed to wastewater treatment: e.g. urban pollution (Shutes, 2001; Lancaster et al., 2004), agricultural wastewaters (Hov & Walseng, 2003; Hansson et al., 2005) and mining drainages (Johnson & Hallberg, 2005; Ji et al., 2007). The popularity of wetland treatment construction is due its ability to capture chemicals, particularly nutrients but also heavy metals, and to their low cost and easily operated and maintained (Mitsch & Gosselink, 2000; Sheoran & Sheoran, 2006; Merricks et al., 2007; Imfeld et al., 2009).

In this case we have focused our attention in ponds constructed during reclamation activities in opencast coal mines to retain the runoff generated in the reclaimed surfaces. The coal production is worldwide increasing (e.g. in 2011 was a 5% higher than 2010) and it is expected that coal continue playing a significant role against the background of the continued rise in global primary energy consumption (BP, 2012; German Natural Resources Agency, 2012). Thus, a probably result of the expansion of coal mining could be the increase in the number of these manmade ponds; about which, as far as we know, scarce information exists. The main objective of these manmade ponds is to avoid the pollution of natural ecosystem downstream reclaimed mines. Additionally, these manmade ponds may provide interesting ecological functions in a context of global lost of natural lentic ecosystems but depending on their environmental characteristics. Heavy metal pollution is one of the most significant environmental challenges facing the mining industry worldwide (Johnson, 2003; Blodau, 2006; Sheoran & Sheoran, 2006) even when opencast coal mines were reclaimed (Hartman et al., 2005; Hopkins et al., 2013). Consequently, the manmade ponds constructed in reclaimed coal mines may be affected by metal pollution which could impair the evolving of the aquatic communities (Merricks et al., 2007; Loayza-Muro et al., 2010) and determine the socio-economic uses (Jurdi et al., 2002; Awofolu et al., 2005; Doupé & Lymbery, 2005).

Despite the increasing popularity of pond creation, little is known about the ecological value or the characteristics of these new sites (Williams et al., 2008; Ruhí et al., 2009).

Moreover, the number, ubiquity and scatter of manmade ponds across many landscapes makes manmade pond useful places in which to investigate fundamental ecology (Wilbur, 1997; De Meester et al., 2005; Oertli et al., 2009). In this work, we are going to focus our study on the primary succession of macroinvertebrate community in manmade ponds. Macroinvertebrates is one of the most frequently used communities in the study of aquatic ecosystems, likely because they play an important role in the ecological functioning of water bodies (Covich et al., 1999; Balcombe et al., 2005; Mermillod-Blondin, 2011). Moreover macroinvertebrate requirements inform about the environmental conditions of the habitat where they were found (Wissinger, 1999; US EPA, 2002a; Stewart & Downing, 2008). Therefore, the study of the macroinvertebrate community offers usefull information about the proceses occuring during primary succession. Succession, or the process describing the change in taxonomic composition in an ecosystem over time, is a key process in ecology related to ecosystem functioning (Margalef, 1968; Odum, 1969; Gutiérrez & Fey, 1980). Most of the studies about succession are based on temporary wetlands (e.g. Lake et al., 1989; Boix et al., 2004; Jeffries, 2010) and, therefore, consider secondary succession (change of taxa after a perturbation). Thus, knowledge about the processes driving communities during primary succession in wetlands and ponds remain limited, particularly with respect to changes in the faunal community (Noon, 1996; Batzer et al., 2006). Recently, primary succession of macroinvertebrate community on permanent manmade water bodies has begun to be studied (Zedler & Callaway, 1999; Ruhí et al., 2009, 2012). Particularly, manmade ponds represent an excellent scenario for analyzing the primary successional process because there were not a previous aquatic community and the age is usually known (Velasco et al., 1993; Flory & Milner, 2000; Céréghino et al., 2008b; Matthews et al., 2009). Explaining the patterns observed in nature is a central aim in ecology and requires the understanding of the causal mechanisms (Townsend & Hildrew, 1994; Weiner, 1995; Verberk et al., 2008). Primary succession is a particularly complex process. Numerous factors including site area, disturbance regimen, habitat heterogeneity, landscape connectivity, source of organisms and biotic interactions, may influence the evolving of manmade ponds (Zedler, 2003; Hansson et al., 2005; Takamura et al., 2008). Therefore, the simultaneous study of abiotic and biotic factors must be considered in order to have a whole vision about the ecosystem changes. Moreover, it has been noted that the contribution of biotic and abiotic factors to the explanation of macroinvertebrate succession varies in importance over time. Three successional phases had been described in temporary ponds (Lake et al., 1989; Boix et al., 2004), but recently also in permanent ponds (Ruhí et al., 2012). The first phase is allogenic as a direct consequence of the filling or the creation of the wetland while the second phase is autogenic because the community changes were not related to the environment since the pond characteristics did not significant change. The third phase in temporary wetlands is allogenic because the drying of the wetland, but in permanent wetlands is autogenic because the environmental conditions did not suffer important changes. Additionally, an important part of the debate about community assemblages have focused on the relative role of deterministic and historical contingent forces (Samuels & Drake, 1997; Fukami & Wardle, 2005; Lepori & Malmqvist, 2009). Deterministic forces are related to predictable trajectories which suggest that under similar conditions the communities of different sites are going to develop common structures (Clements, 1916; Samuels & Drake, 1997; Fukami & Wardle, 2005). In contrast, the stochastic arrival of organisms and the consequent biotic interactions can cause divergences in the community structure among sites with similar conditions (Diamond, 1975; Law & Morton, 1993; Gleason et al., 2003; Fukami & Wardle, 2005).

Therefore, to study the physicochemical and macroinvertebrate community changes over time in manmade ponds constructed in reclaimed opencast coal mines is an important question to solve from ecologic and socio-economic perspectives.

AIMS AND THESIS OUTLINE

AIMS AND THESIS OUTLINE

The main aim of this PhD dissertation was the long-term study of primary succession of permanent manmade ponds created in reclaimed opencast coal mines. Particularly we were interested in:

- Describe the environmental characteristics of constructed ponds of different ages (Chapters 1 and 2)
- Explain the primary succession of macroinvertebrate community in the manmade ponds
    - describe the changes of macroinvertebrate biodiversity over time (Chapter 2)
    - evaluate the factors with more influence on biodiversity changes with especial focus on pond age (Chapter 2)
    - examine what kind of forces, deterministic or historical contingent, have more relevance in the community configuration (Chapters 2 and 3)
    - evaluate the functional and taxonomic trends of macroinvertebrate community during primary succession (Chapter 3)
- Assess the effectiveness of coal mine reclamation:
    - in the control of metal pollution over time:
        - compare the environmental characteristics of manmade ponds of reclaimed mines to the pit-lake located in an un-reclaimed mine (Chapter 1)
        - check if heavy metals were in toxic concentrations to the aquatic community (Chapter 1)
    - and through the biodiversity evaluation of the macroinvertebrate community in the manmade ponds (Chapter 2)

CHAPTERS AND PUBLICATIONS

CHAPTERS AND PUBLICATIONS

This Ph.D dissertation is based on three studies which are integrated in three papers submitted to scientific journals:

Study 1. Assesing metal pollution in ponds constructed for controlling runoff from reclaimed coal mines

Leticia Miguel-Chinchilla, Eduardo González, Francisco A. Comín

Status: In review; Wetlands

Study 2. Macroinvertebrate biodiversity patterns during primary succession in manmade ponds

Leticia Miguel-Chinchilla, Dani Boix, Stephanie Gascón and Francisco A. Comín

Status: Submitted to Hydrobiologia

Study 3. Taxonomic and functional successional patterns of poorly and easily dispersing macroinvertebrates: a case study from isolated manmade ponds at reclaimed opencast coal mines

Leticia Miguel-Chinchilla, Dani Boix, Stephanie Gascón and Francisco A. Comín

Status: Submitted to Journal of Environmental Management.

Each chapter has its own introduction, results and discussion it also includes a section explaining the specific methodology applied in each chapter.

GENERAL METHODS

STUDY SITE

To achieve our objectives we studied the manmade ponds constructed in reclaimed opencast coal mines located in the Teruel coalfield, a mountainous area in northeastern Spain (Fig. 1). This is a region characterized by a continental Mediterranean climate with two rain periods in spring and autumn, long and cold winters and hot and short summers. This windy area has a high inter and intra-annual variability with dominance of dry years and its characterized by the dominance of limestone substrates.

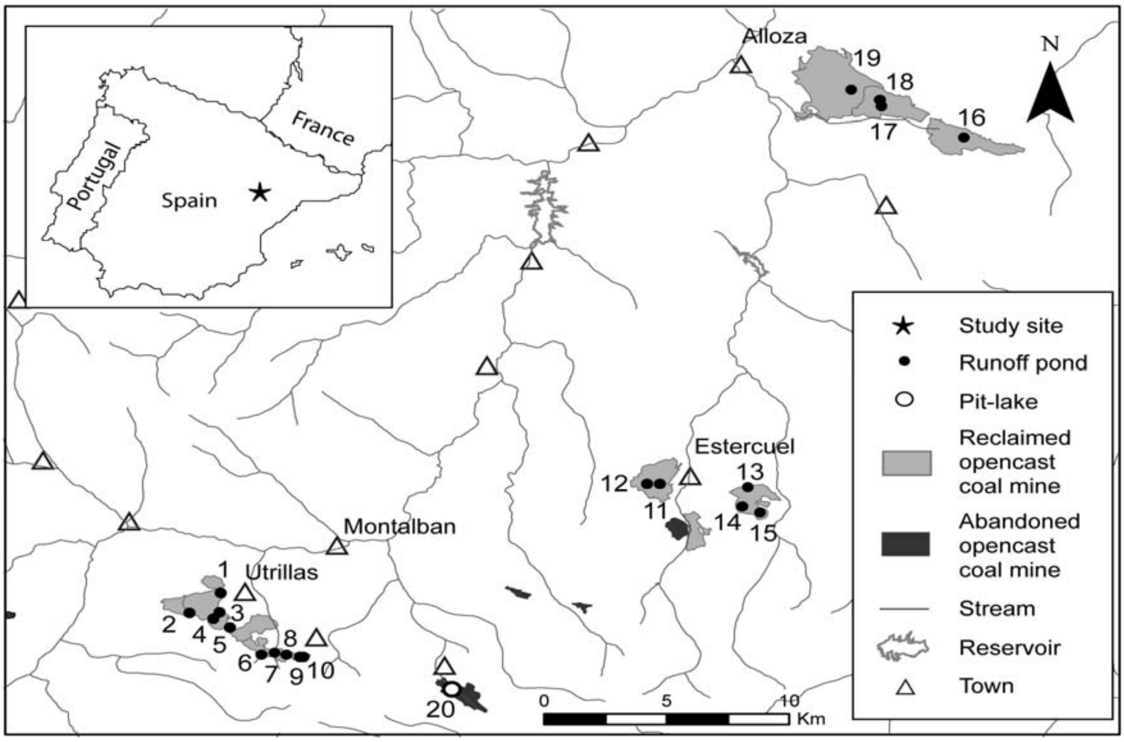

Figure 1: Study site

In this area, mining worked the same geological coal seam and surface mining used similar extraction methods. First, coal extraction requires the construction of an external dump next to the excavating area. As mining advances through the coalfield along the coal seam, the overburden is used to refill the previously mined pits and the post-mining topography is created according to the reclamation objectives. The main aim of mining reclamation was minimize runoff and sediment export to downstream ecosystems. The control of the offsite effects was conducted by the design of stable relief forms and safety structures such as ponds that guarantee the non-emission of sediments and contaminants from reclaimed sites to adjacent natural watercourses. Complementary, the post-mining landscapes in this area were mainly designed to support grassland and farming platforms.

The studied manmade ponds were located in mines where predominate integrated shapes with soft slopes that contribute to the production of functional systems, but in some cases we can also found slopes of near 30º and rectilinear shapes (Nicolau, 2003). The overburden material removed during surface mining and used to refill the open pits were characterized by massive structure because of their very low organic matter and high silt and fine sand contents (Nicolau, 2002). Reclaimed topsoil also showed low organic matter content but had more balanced particle size distribution, greater stoniness and low bulk density (Nicolau, 2002). The revegetation of the constructed slopes was undertaken by sowing a mixture of perennial grasses and leguminous herbs and occasionally trees (mainly pines) were included (Espigares et al., 2011). In spite of reclamation activities, nowadays we can found eroded slopes with low density vegetation (favored by sheep grazing) that, in some cases uncover the overburden substratum (Martínez Pantaleón, 1999; Nicolau, 2002).

The manmade ponds considered to this study were constructed to manage the water runoff produced in the reclaimed mines to avoid contaminating natural ecosystems. The manmade ponds were designed following natural ecosystems so they would not require permanent human management (see pictures 2 to 18 from Appendix 1). After a preliminary study of the manmade ponds located in the three mining areas we selected 19 manmade ponds, covering an age range of 22 years that were as similar as possible in their environmental characteristics. Ponds had different ages because exploitation and reclamation of opencast coal mines took place in different years. The manmade ponds were oval shaped, and had similar size (approx. 2.5 ha on average). Littoral vegetation was dominated by *Typha* sp. distributed in an average band of approx. 1.5 m around the pond. All ponds were permanent and endorheic; except for two ponds that were connected to a small stream (6 and 7 in Fig. 1), the water arrived from superficial and sub-superficial runoff during rain events. This small stream rise close to mining area and only have exit to natural ecosystems when the water in the second pond exceeds its storage capacity. Water level fluctuations were similar in all the ponds because they were mainly related to the rain events. Finally, it is important to note that no natural ponds and wetlands were found in the river basin where the mines are located.

In addition to the 17 runoff ponds, we sampled one pit-lake from an un-reclaimed coal mine within the same study area (picture 19, Appendix 1). Pit-lakes, which are formed when water fills excavated mining pits, are typically deep lakes with vertical walls. The pit-lake surface was 8 ha, and the pit-lake was narrow, deep, without a littoral zone and vegetation and enclosed by steep rock walls averaging 10 m high. The low water level and steep banks, typical characteristics of the un-reclaimed pit-lakes in the study area, make sampling extremely difficult and dangerous, and these conditions prevented us from including other pit-lake replicates in the study. The sampled pit-lake was older than 25 years old.

SAMPLING AND SAMPLE PROCESING

Two surveys were conducted in the 19 manmade ponds and the pit-lake in spring and in summer of 2009. In each pond and in the pit-lake, samples of macroinvertebrates, water and sediment were collected, and pond and landscape characteristics were measured. *Typha* sp. samples were collected only in summer 2009. A summary of the environmental variables considered by chapters is shown in table 1.

Temporal approximation

The consideration of long periods of time is necessary to understand the evolving of permanent manmade ponds (e.g. Petranka et al., 2007; Moreno-Mateos et al., 2012; Ruhí et al., 2012). Particularly, in order to understand the whole successional sequence during primary succession and the changes of the community assemblage, long observational periods are required (Barnes, 1983; Guo, 2003; Spieles et al., 2006). Due to the difficulty of obtaining long-term series of data, the use of chronosequences have been frequently used (Bossuyt et al., 2003; Marchetti et al., 2010; Hart & Davis, 2011; Helsen et al., 2012). Chronosequence is a space-for-time substitution approach used to the study of long-term processes appropriate when sites differing in age were created in similar conditions and were influenced by similar factors (Majer & Nichols, 1998; Walker et al., 2010).

The age of each pond was calculated based on the date of the end of mining exploitation. Thus, ponds number 11, 12, 14 and 15 were approx. 1 year old, pond number 13 was approx. 3 years old, pond 16 was approx. 6 years old, pond 4 was approx. 9 years old, ponds 8, 9 and 10 were approx. 10 years old, pond 19 was approx. 11 years old, pond 17 and 18 were approx. 13 years old, pond 3 was approx. 15 years old, pond 2 was approx. 16 years old, ponds 5, 6 and 9 were approx. 19 years old and pond 1 was approx. 22 years old.

Water characteristics

Dissolved oxygen, temperature, conductivity and pH were measured *in situ* using portable probes (WTW Multiline P4, Weilheim, Germany). Water was collected at a depth of 15-20 cm and brought to the lab for further analyses. Chlorophyll-*a* was analyzed by spectrophotometry (HeλIOS α , Thermo Electron Corporation, US). Alkalinity (mg l^{-1}) was determined by pH potentiometric automatic titration with 0.004 N H_2SO_4 (Metrohm, Herisau, Switzerland). Water samples were filtered through pre-ashed glass-fiber filters. Total suspended solids and total suspended organic matter were calculated by filter and burned filter weight differences, respectively. Dissolved SO_4^{2-} (mg l^{-1}) was determined by ion chromatography (Metrohm 861 Advanced Compact IC, Herisau, Switzerland). Total dissolved nitrogen (mg l^{-1}) and non-purgeable organic carbon (mg l^{-1}) were determined by catalytic combustion at high temperature using a Multi-N/C 3100 analyzer (Analytik Jena, Jena, Germany). In addition, we determined total dissolved phosphorus and 10 dissolved trace metals (mg l^{-1}), Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn, using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES iCAP6300Duo; Thermo Fisher, Waltham, MA, USA). Analyses performed in the lab followed the standard methods of the American Public Health Association (APHA et al., 1992). All the materials used for metal analysis were soaked in 10% HNO_3 (Sastre et al., 2002).

Sediment characteristics

One composite sample of sediment was collected in the littoral zone of each pond. Sampling was limited to the superficial sediment (i.e., top ~10 cm) because most of the physical and chemical changes in sediments occur within the upper 10 cm layer (Meyer et al., 2008). Sediment pH and conductivity (µS cm^{-1}) were measured in a solution of 10 g of fresh sediment dispersed in deionized water (pH: 2.5:1 g ml^{-1}, conductivity: 5:1 g ml^{-1}) after shaking for 30 min. The collected sediments were air dried and sieved into fractions. The < 2 mm fraction was used to determine the particle-size by laser-diffraction analysis (Syvitski, 2007) using a Mastersizer 2000® particle size analyzer (Malvern Instruments, Malvern, UK). We obtained the percentage of sand, thick silt, thin silt and clay. The <2 mm fraction was also used to determine total carbon (%), total inorganic carbon (%) and total sulfur (%) using an elemental analyzer (LECO SC-144DR; Leco Instruments, St Joseph, MI, USA). Total organic carbon (%) was calculated by the difference between total carbon and total inorganic carbon. Total nitrogen (%) was determined using a Vario Max elemental analyzer (Elementar, Harnau, Germany). The <63 μm fraction was used to determine total phosphorus (mg kg^{-1} DW) and total and extractable heavy metals (mg kg^{-1} DW): Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. The phosphorus and metals were analyzed per the US Environmental Protection Agency methods (US EPA, 2007), and the extractable metals were obtained using acetic acid (Davidson et al., 1994; Rauret, 1998; Pueyo et al., 2001). In both cases, sediment digestion was performed by microwave extraction (Speedwave MWS-3, Berghof, Germany). Total phosphorus and total and extractable metal content were determined using the ICP-OES following the American Public Health Association methods (APHA et al., 1992).

Vegetation (*Typha* sp.)

Three plants of *Typha* sp. of more than 1.5 m high and without inflorescence were collected at each manmade pond. Plants were carefully washed using tap and distilled water, then cleaned by immersion in 0.01 M ethylenediaminetetraacetic acid (EDTA) to remove any absorbed metals and finally rinsed with deionised water (Carranza-Alvarez et al., 2008). Later, the plants were dried to constant weight at 60ºC. The metal concentration in the tissues of *Typha* sp. varied, with root > rhizome > leaf (Dunbabin & Bowmer, 1992; Sasmaz et al., 2008), so the dried plants were separated into leaf, root and rhizome and grounded in a laboratory mixer mill (MM400 Retsch, Haan, Germany). The plant material was digested with nitric acid to solubilize metals (Meeravali & Kumar, 2000; Sastre et al., 2002) using a microwave system to reduce the total analysis time and the risk of sample contamination (Nadkarni, 1984; Smith & Arsenault, 1996; Sastre et al., 2002). Finally, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were determined in ICP-OES following APHA et al. (1992) methods. The concentration of all metals in the plant tissues was expressed in mg kg^{-1} DW.

Landscape characteristics

Pond and landscape characteristics were estimated with geographic information systems (ArcGis 9.3.1 ESRI Inc, Redlands, CA, USA) and fieldwork observations. We calculated pond size (m^2), pond littoral slopes (%), pond basin slope (degrees), vegetated pond littoral area (m^2), nearest pond distance (m^2), nearest river distance (m^2) and the numbers of ponds and rivers in a 1000-m buffer.

Macroinvertebrate sampling

Macroinvertebrate collections were restricted to shallow sites (<1 m deep) located in the littoral of the manmade ponds. One integrated sample from the identified major habitats (emergent plants, bottom and water column) was collected from each pond with a 250 μm mesh hand-net using the kick-and-sweep sampling technique. Sampling was considered complete when no new taxa were found by visual observation. The macroinvertebrate samples were preserved in 4% formalin. The samples were washed through nested sieves in the lab and the collected fauna was sorted under a stereomicroscope and were identified mostly to the genus level (except for the Oligochaeta, Ceratopogonidae and Dolichopodidae, which were identified to the family level and the Chironomidae, which were identified to the subfamily and tribe level).

Table 1: Summary table of environmental variables and units considered by chapter.

Variable	Units	Chapter	
		1	2
Water			
Dissolved oxygen	mg l^{-1}		X
Temperature	ºC		X
Conductivity	µS cm^{-1}	X	X
pH		X	X
Alkalinity	mg l^{-1}	X	X
Chlorophyll-a	mg l^{-1}		X
Total suspended solids	mg l^{-1}		X
Total suspended organic matter	mg l^{-1}		X
SO_4^{2-}	mg l^{-1}	X	
Non purgueable organic carbon	mg l^{-1}		X
Total dissolved nitrogen	mg l^{-1}		X
Total dissolved phosphorus	mg l^{-1}		X
Dissolved Al	mg l^{-1}	X	X
Dissolved As	mg l^{-1}	X	X
Dissolved Cd	mg l^{-1}	X	X
Dissolved Cr	mg l^{-1}	X	X
Dissolved Cu	mg l^{-1}	X	X
Dissolved Fe	mg l^{-1}	X	X
Dissolved Mn	mg l^{-1}	X	X
Dissolved Ni	mg l^{-1}	X	X
Dissolved Pb	mg l^{-1}	X	X
Dissolved Zn	mg l^{-1}	X	X
***Typha* sp.**			
Al	mg kg^{-1} DW	X	
As	mg kg^{-1} DW	X	
Cd	mg kg^{-1} DW	X	
Cr	mg kg^{-1} DW	X	
Cu	mg kg^{-1} DW	X	
Fe	mg kg^{-1} DW	X	
Mn	mg kg^{-1} DW	X	
Ni	mg kg^{-1} DW	X	
Pb	mg kg^{-1} DW	X	
Zn	mg kg^{-1} DW	X	
Landscape			
Pond area	m^2		X
Pond littoral slope	**%**		X
Pond basin slope	degrees		X
Vegetated pond littoral area	m^2		X
Nearest pond distance	m		X
Nearest river distance	m		X
Number of ponds in a 1000-m buffer			X
Number of streams in a 1000-m buffer			X

Variable	Units	Chapter	
		1	2
Sediment			
Conductivity	µS cm^{-1}	X	X
pH		X	X
Total Carbon	%		X
Total inorganic carbon	%		X
Total organic carbon	%		X
Total sulfur	%	X	X
Total Nitrogen	%		X
Total Phosphorus	mg kg^{-1}		X
Total Al	mg kg^{-1}	X	X
Total As	mg kg^{-1}	X	X
Total Cd	mg kg^{-1}	X	X
Total Cr	mg kg^{-1}	X	X
Total Cu	mg kg^{-1}	X	X
Total Fe	mg kg^{-1}	X	X
Total Mn	mg kg^{-1}	X	X
Total Ni	mg kg^{-1}	X	X
Total Pb	mg kg^{-1}	X	X
Total Zn	mg kg^{-1}	X	X
Extractable Al	mg kg^{-1}	X	
Extractable As	mg kg^{-1}	X	
Extractable Cd	mg kg^{-1}	X	
Extractable Cr	mg kg^{-1}	X	
Extractable Cu	mg kg^{-1}	X	
Extractable Fe	mg kg^{-1}	X	
Extractable Mn	mg kg^{-1}	X	
Extractable Ni	mg kg^{-1}	X	
Extractable Pb	mg kg^{-1}	X	
Extractable Zn	mg kg^{-1}	X	
Sand fraction	%		X
Thick silt fraction	%		X
Thin silt fraction	%		X
Clay fraction	%		X

CHAPTER 1

Assessing metal pollution in ponds constructed for controlling runoff from reclaimed coal mines

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Abstract Constructing ponds to protect downstream ecosystems is a common practice in opencast coal mine reclamation. As these ponds remain integrated in the landscape, it is important to evaluate the extent of the effect of mine pollution on these ecosystems. However, this point has not been sufficiently addressed in the literature. In this paper, we evaluated the concentration of 10 heavy metals in the water, sediment and dominant macrophyte (*Typha* sp.) in 17 manmade runoff ponds at reclaimed opencast coal mine sites. The ponds, which ranged in age from 1 to 19 years old, were located in a coal field in northeastern Spain. To evaluate mining pollution, we used mean metal concentrations in unpolluted sites as well as referenced toxicity levels for aquatic organisms. In addition, we sampled a pit-lake created in an un-reclaimed mine located in the same region. The runoff ponds showed toxic concentrations of Al, Cd, Cu and Ni in the water and As and Ni in the sediment maintained over time. Moreover, the metal concentrations in the macrophytes were higher than the average concentration of metals in plants and the metal concentrations of *Typha* sp. in un-polluted sites. Nevertheless, metal concentrations were lower than those in the pit-lake. This study highlights the importance of mining reclamation in regard to the health of aquatic ecosystems and suggests that the existence of chronic metal toxic levels in the ponds could be jeopardizing pond ecological functions and services.

Key words: runoff control, manmade ponds, heavy metals, reclamation, rehabilitation, coal mining, post-mining landscapes

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INTRODUCTION

Surface coal mining operations remove the soil and rocks over the coal seam (overburden) to gain access to the underlying coal. Despite reclamations, surface mining produces major environmental disturbances due to excavation operations, including deforestation, topsoil loss, topological alterations, soil compaction, hydrological changes and aquatic contamination (Shrestha & Lal, 2006; Cravotta, 2008; Pond et al., 2008; Palmer et al., 2010). Among all disturbances, the heavy metal pollution derived from acidic mine drainages (hereafter called AMD) is one of the greatest threats to the aquatic ecosystems because this factor is degrading to the aquatic habitat and is potentially toxic to aquatic organisms (Dunbabin & Bowmer, 1992; MacDonald et al., 2000; Cravotta, 2008).

The main goal of mine reclamation is to isolate the pollutants from the ecosystems located downstream of the mine sites. This objective should be achieved in combination with the final use of the reclaimed mine (e.g., production of biomass for energy and sustain natural grassland and forest) because the resulting ecosystem will remain integrated in the landscape (Bungart & Hüttl, 2001; Nicolau, 2003; Gould, 2012; Vickers et al., 2012). The control of the offsite effects are addressed by the design of stable relief forms and safety structures, such as wetlands and ponds that guarantee the non-emission of sediments and contaminants from restored sites to adjacent natural watercourses (Sawtsky et al., 2000; Nicolau, 2003; Wong, 2003). These water bodies are normally constructed with two objectives: the control of the runoff generated in the reclaimed mines and the specific treatment of AMD.

The water bodies constructed to retain the runoff may be affected by metal pollution although AMD was not evident. In reclaimed mines, coal mineral could be present in the overburden materials used to reconstruct the topography, and metals could be released from the oxidation of pyrite present in the coal mineral (Johnson, 2003; Sheoran & Sheoran, 2006; Cravotta, 2008; Griffith et al., 2012). The manmade wetlands and ponds remain integrated in the landscape after the end of mining reclamation and may provide new ecological functions and services to society, such aquatic fauna habitats and recreational areas. But these functions are going to depend on the quality of the ecosystem. Therefore, to know the extent to which wetlands and ponds are affected by metal pollution is an important issue that nevertheless was insufficiently addressed in the extant literature.

The main objective of this work was to explore the metal pollution in manmade ponds constructed for runoff-control in reclaimed coal mines (hereafter called runoff ponds). Specifically, we were interested in evaluating the temporal changes in metal concentrations to determine if toxic concentrations of metals were present in the aquatic ecosystem. A chronosequence was used to approximate the temporal study of the metal pollution for runoff ponds. A comprehensive assessment of the runoff ponds was conducted through a study of water, sediment and aquatic macrophytes, which are three main factors involved in the process of heavy metal removal (Dunbabin & Bowmer, 1992; Sheoran & Sheoran, 2006).

METHODS

Sample collection and processing

In this study we used the physicochemical data from 17 of the 19 manmade ponds located in reclaimed mines and the pit-lake located in the un-reclaimed mine. We discarded two manmade ponds (4 and 5, Fig 1) in this study because *Typha* sp. was absent.

In water we analyzed conductivity, pH, alkalinity, SO_4^{2-} and dissolved concentration of Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. In sediment, pH, conductivity, total sulfur and total and extractable heavy metals (the same ones analyzed in water) were considered. Finally, we also analyzed the concentrations of the 10 heavy metal in *Typha* sp. tissues (root, rhizome and leaf). Sample collection and processing and the variables used in this chapter were described in the general methods section.

Data analyses

Water and sediment

Changes in the water and sediment characteristics in the runoff ponds were assessed over time by linear mixed-effect models (LMM). The LMM allowed for the detection of trends in the change of the environmental variables and control for the effect of the sampling season. For each variable, we built a model in which the fixed factor was the age of the ponds, and sampling season was used as random variable. LMM analyses were conducted with R software v.2.15.1 (R Core Team, 2012). We used the function "lme" implemented in the "nlme" package (Pinheiro et al., 2012). The "lme" function assumes that both the random effects and the errors follow normal distributions. Water and sediment variables were transformed with the log(x+1) to improve model fitting.

The metal toxicity in the runoff ponds was evaluated using several criteria obtained from the literature. To evaluate water pollution, we used the criteria established by the Environmental Protection Agency of United States for the indefinite (CCC: Criterion Continuous Concentration) and brief (CMC: Criteria Maximum Concentration) exposure of aquatic organisms without producing unacceptable effects (US EPA, 2002b). In addition, we used the toxic concentration of dissolved metals to plants defined by Markert (1992). The sediment metal pollution was evaluated using the quality guidelines for freshwater ecosystems proposed by MacDonald et al. (2000) that provide an accurate basis to predict the absence of sediment toxicity (TEC: Threshold Effect Concentration) and the presence of sediment toxicity (PEC: Probable Effect Concentration). Finally, the metal concentrations in the runoff ponds were compared to the metal concentration in the pit-lake created within the un-reclaimed coal mine.

Aquatic macrophytes

We assessed the influence of mining on the macrophytes by comparing the metal concentration of the *Typha* sp. to the average concentration of metals in plants following Markert (1992). We did not use a chronosequence approach to analyze metal content in *Typha* sp. because the time that each plant was exposed to the metals in the runoff pond could not be determined.

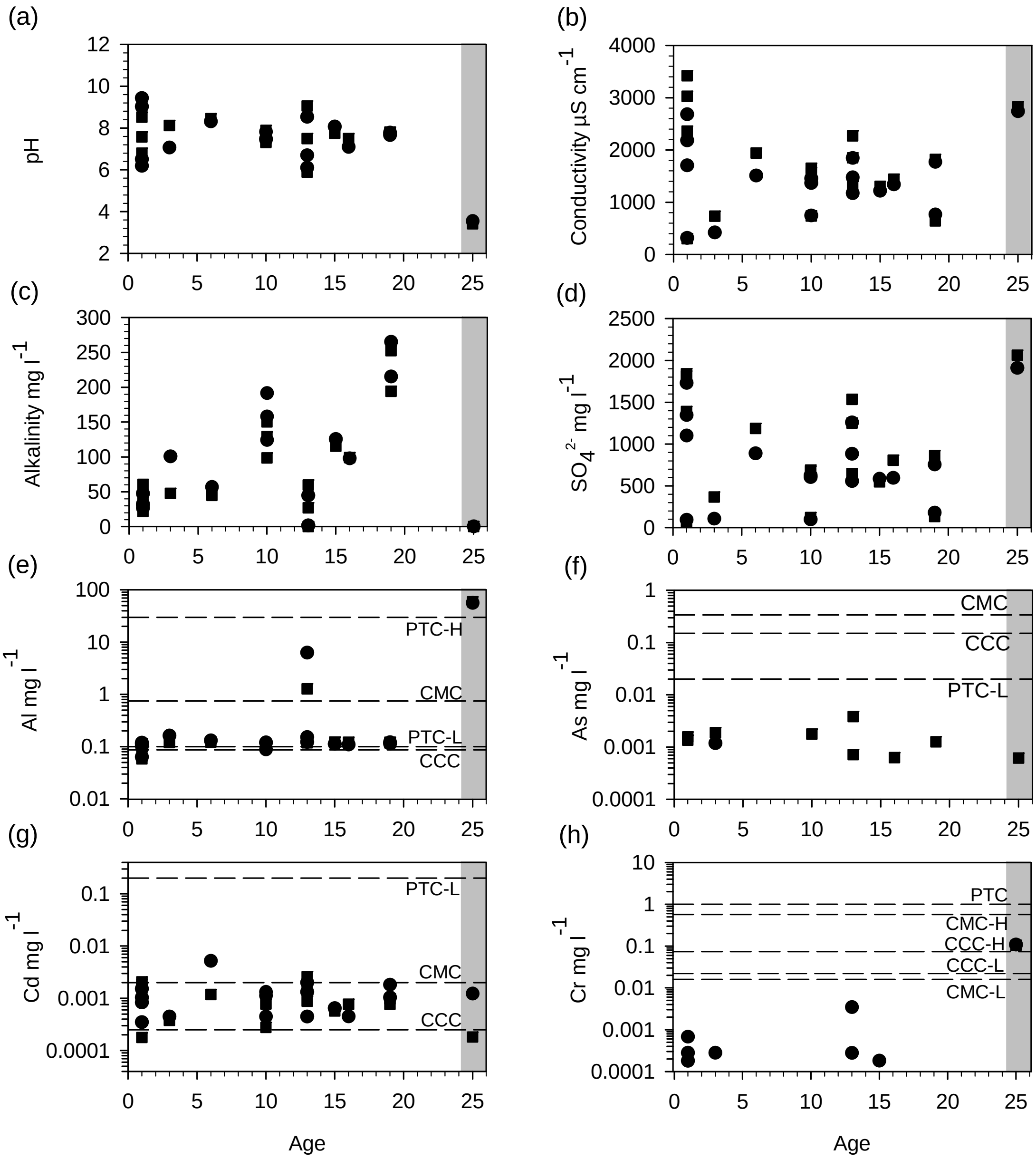

Figure 2: Scatter plots of the water characteristics in relation to the runoff pond age. Circles represent spring data, and squares represent summer data. A black continuous line was drawn when a LMM could be fitted. In that case, t and p-values were shown. The pit-lake values were shown in the grey area of the graphic. Dashed lines indicate reference criteria to metal pollution CMC: Criteria Maximum Concentration (US EPA, 2002b) CCC: Criterion Continuous Concentration (US EPA, 2002b); PTC: average toxic concentration to plants (Markert, 1992). H represents the high value, and L represents the low value when a range of references exists.

RESULTS

Metal pollution evaluation

The water in the runoff ponds had a neutral pH (7.67 ± 0.16). The water pH in the pit-lake was acidic (3.48 ± 0.06), and alkalinity was not detected. Except for similar concentrations of As and Cd, the concentrations of sulfate and heavy metals in the runoff ponds were several orders of magnitude lower than the pit-lake (Fig. 2 from a to n). According to the criteria proposed by the US EPA (2002b), the dissolved Al, Cd, Cu and Ni mean concentrations in the runoff ponds were from one and six times greater than the CCC criteria, and only the Cu concentrations were above CMC criteria. The pit-lake showed Al, Cd, Cr, Cu, Ni, Pb and Zn metal concentrations above CCC criteria and Al, Cu and Zn concentrations above CMC criteria. The differences between the pit-lake and reference

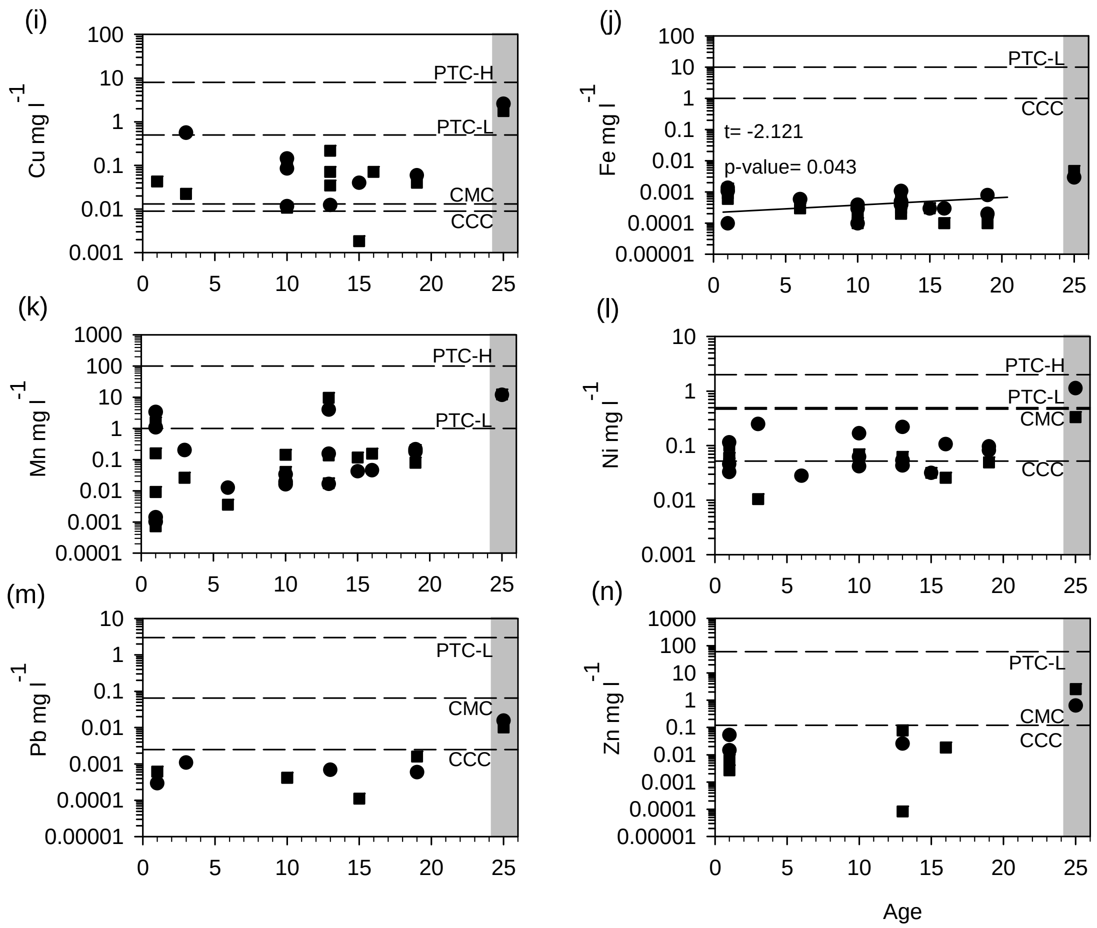

Figure 2 (Continuation)

levels were higher compared with the runoff ponds (Fig. 2 from e to n); notably, the Al and Cu metal concentrations in the pit-lake were more than 100 times greater. Only Al in the pit-lake (Fig. 2e) exceeded the toxicity values established for plants in Markert (1992).

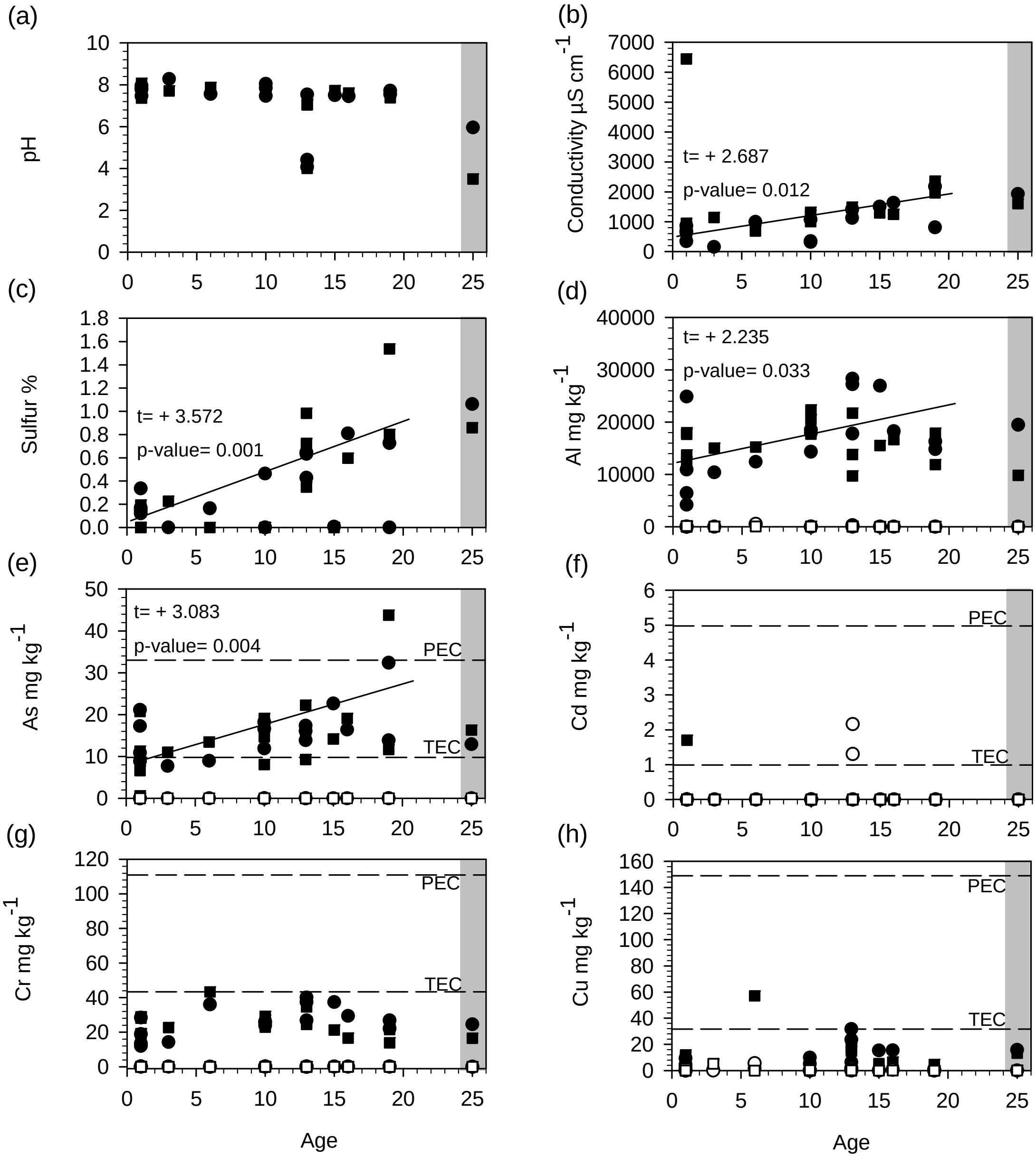

Figure 3: Scatter plots of the sediment characteristics in relation to the runoff pond age. Circles represent spring data, and squares represent summer data. For the heavy metals, the black circles and squares represent total concentration, and the white circles and squares represent extractable concentrations. A black continuous line was drawn when a LMM could be fitted. In that case, t and p-values were shown. The pit-lake values were shown in the grey area of the graphic. Dashed lines indicate reference criteria to metal pollution TEC: threshold effect concentration and PEC: probable effect concentration (MacDonald et al., 2000).

In the sediment, the pH was neutral in the runoff ponds (7.32 ± 0.19) and acidic (4.73 ± 1.23) in the pit-lake, and the total sulfur was three times higher in the pit-lake than the runoff ponds. Despite these results, we did not detect differences in the total and extractable heavy metals among runoff ponds and the pit-lake (Fig. 3 from a to m). In both water bodies, only As and Ni exceeded the quality guidelines established by McDonald et al. (2000) for sediments in freshwater ecosystems. Total As was 1.5 times higher than the threshold value of TEC criteria, and Ni was 8 times higher than TEC and approximately 3 times higher than PEC in both the runoff ponds and the pit-lake.

The *Typha* sp. in almost all runoff ponds exceeded the average metal concentration of Al, Fe and Ni in the root and rhizome and of As in the roots only (Fig. 4 a, b, f and h). The average concentration of these metals in the tissues of *Typha* sp. were 2 and 8 times higher than mean levels, but the Fe concentration in the roots was approximately 40 times higher. The mean concentrations of Cd, Cr, Cu, Pb and Zn in plants were only above acceptable limits in one or two ponds,

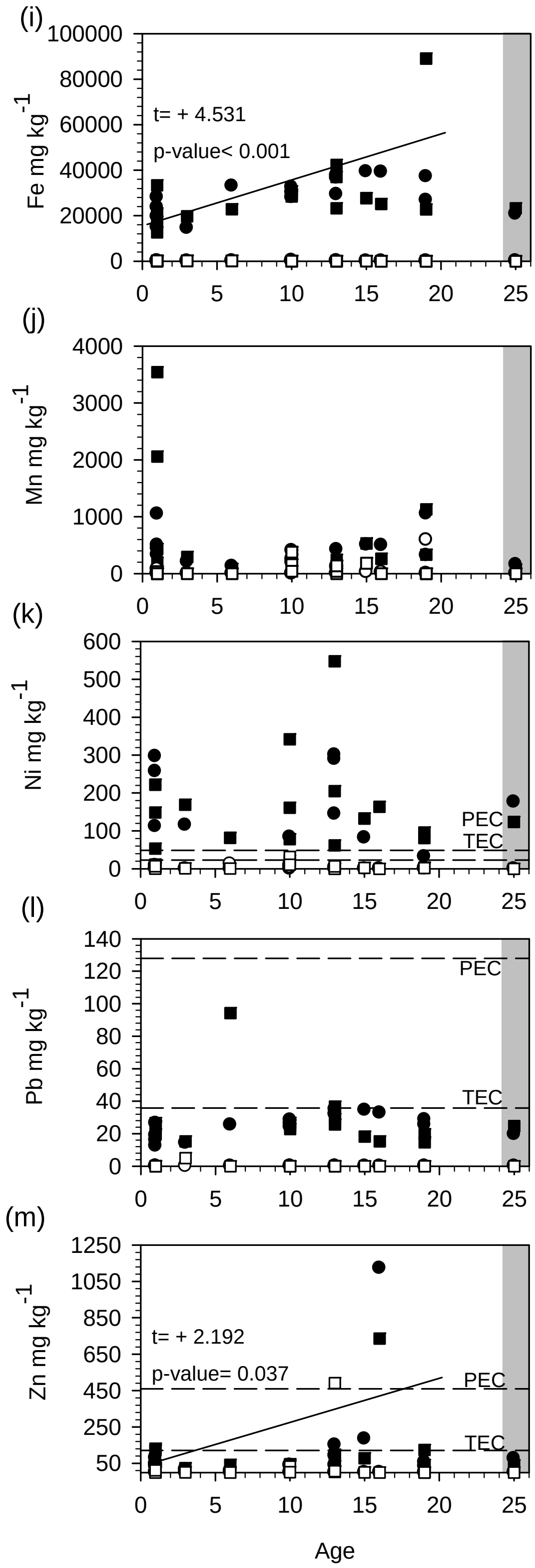

Figure 3 (Continuation)

primarily in the underground parts of the *Typha* sp., although Mn levels in leaves were higher than the mean plant concentrations in more than 50% of the runoff ponds (Fig. 4 c, d, e, g, i and j).

Changes over time

Runoff ponds had similar values in the water characteristics regardless of age differences. Only the Fe concentration decreased over time in the water (significant LMM, Fig. 2j). The conductivity, total sulfur, Al, As, Fe and Zn in the sediment increased with pond age (significant LMM, Fig. 3 b, c, d, e, i and m). Toxic levels of As in the sediment to the aquatic organisms were maintained above TEC and below PEC thresholds, although the concentrations increased over time.

DISCUSSION

Ponds exhibit toxic metal concentrations for aquatic life

The references used to detect metal toxicity in the aquatic organisms showed potentially toxic levels of Al, Cd, Cu and Ni in water and As and Ni in sediment (MacDonald et al., 2000; US EPA, 2002b). Ponds are generally considered to be good metal sinks (Dunbabin & Bowmer, 1992; Mitsch & Wise, 1998; Sheoran & Sheoran, 2006; Merricks et al., 2007). However, the case of the Teruel coalfield, however, showed that the pond's ability to retain metals in the sediment did not reduce the water's metal concentration below levels toxic to the aquatic organisms. CCC, CMC, PEC and TEC criteria consider the negative effects that heavy metals could cause for a wide range of aquatic organisms (plankton, macroinvertebrates and fishes). Therefore, the persistent metal concentration above toxic levels over time may compromise the composition and development of the entire aquatic community. For example, metal pollution in aquatic sites could reduce the taxonomic richness of macroinvertebrate communities and induce a shift toward more tolerant taxa (Clements, 1994; David, 2003; Merricks et al., 2007; Loayza-Muro et al., 2010). In fishes, heavy metals may restrict the presence of some species, reduce embryonic survival and increase the frequency of body malformations and deaths (Dubé et al., 2005; Lindberg et al., 2011; Witeska et al., 2013).

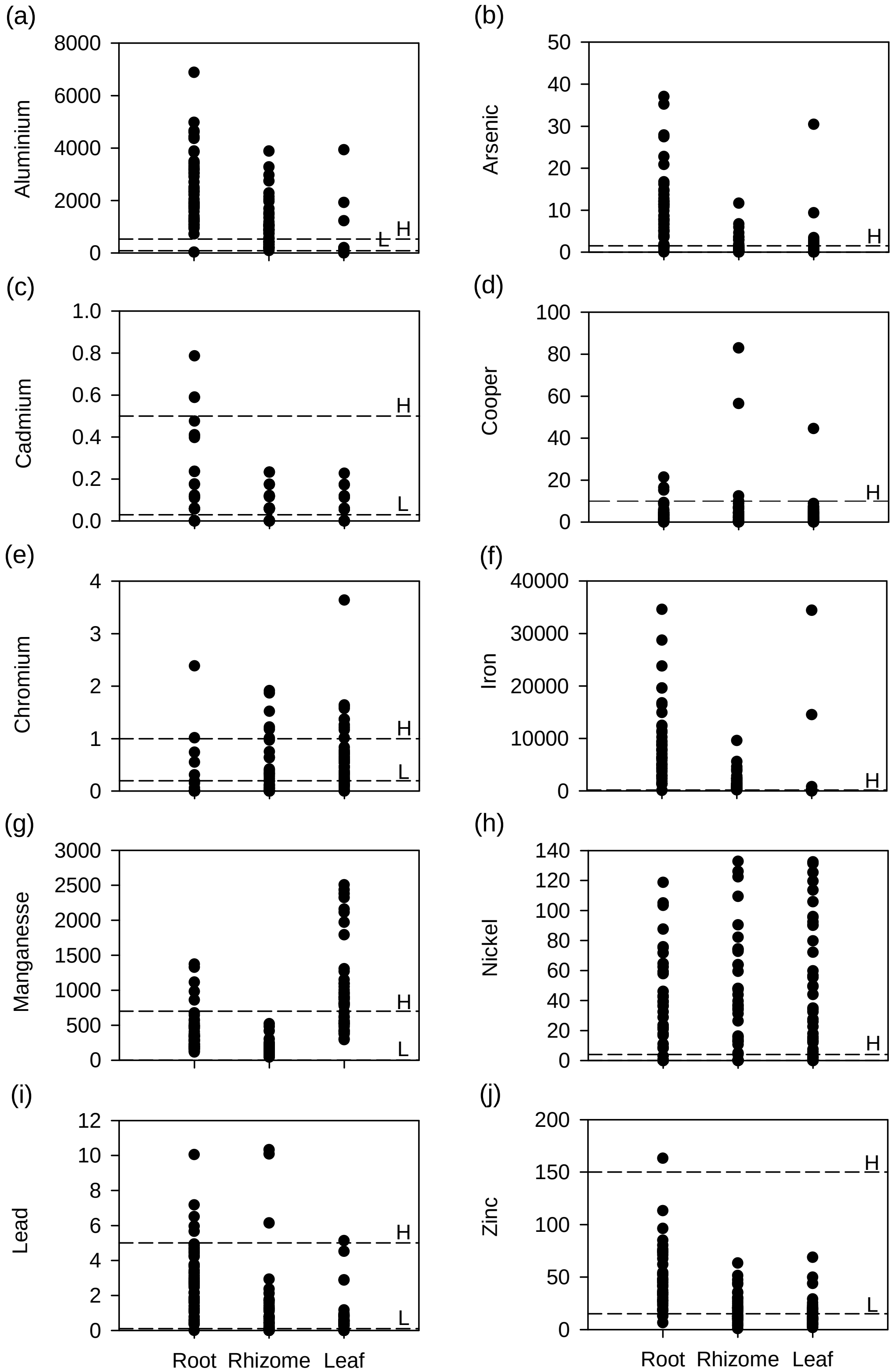

Figure 4: Metal concentration in *Typha* sp. root, rhizome and leaf tissues. Dashed lines indicate the average range of metal concentrations for plants (Markert, 1992). H indicates the high value, and L indicates the low value of the average range when a range of references exists. The data are expressed in mg kg^{-1} DW.

Although metal concentrations in the runoff ponds were not above levels considered to be toxic to plants (Markert, 1992), the negative effects of the high metal concentrations were reflected in the study of the *Typha* sp. We detected Al, As, Fe, Mn and Ni concentrations in the *Typha* sp. that were well above concentrations considered to be normal for plants (Markert, 1992) and higher than the metal concentration of *Typha* sp. growing in non-polluted sites (Babcock et al., 1983; Samecka-Cymerman & Kempers, 2001). *Typha* sp. is known to accumulate high amounts of metals in their tissues (McNaughton et al., 1974; Ye et al., 2003; Demirezen & Aksoy, 2004), and our results agree with these previous findings. Remarkably, *Typha* sp. was almost the only macrophyte in the runoff ponds. This observation suggests that other plants were less competitive or unable to exist due to prevalent heavy metal levels in the runoff ponds, and ponds ultimately suffered from undesirable heavy metal pollution.

The runoff ponds had higher concentrations of heavy metals in both water and sediment compared with the streams located in the same river basin, evidencing the effect of metal pollution from coal mining. Only Cr and Fe dissolved in the water of the runoff ponds were lower than the concentrations in the adjacent streams, while dissolved Pb in the water and total Cd in the sediment showed similar values in both aquatic ecosystems (Miguel-Chinchilla, L., unpublished data).

Metal pollution persists in runoff ponds over time

The temporal approach used in this study indicates that metal pollution in the runoff ponds persisted even 19 years after their construction. The increase of total sulfur, Al, As, Fe and Zn in sediments over time may evidence a continuous introduction of metals into the runoff ponds. When coal mine spoils are exposed to natural weathering conditions, the pyretic minerals contained in the coal are oxidized in presence of oxygen and water, producing sulfuric acid and releasing heavy metals (Johnson, 2003; Sheoran & Sheoran, 2006; Cravotta, 2008). Thus, sulfates and metals could move from the reclaimed mines into the runoff ponds during rain events dissolved in the water runoff. Moreover, soil erosion and overburden during storm events could mobilize sulfur and metals in suspension.

Despite continuous metal introduction into the runoff ponds, the pH, alkalinity, SO_4^{2-} and dissolved heavy metals in water and the extractable metals in sediment did not change over time. These results suggest that during the studied period, the runoff ponds had a chemical equilibrium that may be favored by carbonate parent material that provides extensive internal buffering capacity (Pond et al., 2008; Bernhardt & Palmer, 2011; Griffith et al., 2012).

Relevance of mine reclamation

The concentration of heavy metals in the water of the runoff ponds was significantly lower than the concentration of heavy metals found in the pit-lake located in the un-reclaimed mine. Although the pit-lake was formed in a limestone area more than 25 years ago, it had a low pH and high concentrations of sulfate and heavy metals typical in this type of lakes (Blodau, 2006; Yucel & Baba, 2012). The metal concentrations of most of the studied metals in the water were above the levels considered to be toxic for the aquatic organisms. Moreover, the combination of the acidic pH and the toxic level of Al could contribute to the absence of macrophytes and aquatic plants in the pit-lake (Markert, 1992; Samecka-Cymerman & Kempers, 2001; Brix et al., 2002). Therefore, if opencast coal mines were not reclaimed in our study area, acidic and metal polluted lakes with scarce abilities to sustain a biological community would have formed. In accordance to previous works (Younger, 2001; Rodrigue et al., 2002; Wei et al., 2011), our study highlighted the relevance of opencast coal mine reclamation.

CONCLUSIONS AND MANAGEMENT IMPLICATIONS

Reclamation of opencast coal mines plays a key role to reduce metal pollution and obtain functional ecosystems in post-mining landscapes. Nevertheless, the long-term monitoring of manmade ponds is required because this study reveals that metal pollution in reclaimed coal mines may be a chronic problem.

Runoff ponds are efficient tools to avoid mining pollution of natural ecosystems under the condition that runoff is managed through endorheic basins. Nevertheless, this fact should not justify metal pollution in manmade ponds. The reclamation of opencast coal-mining landscapes should focus on maintaining un-polluted and self-organized ecosystems in combination with the final use of the post-mining area (Hobbs & Norton, 1996). Topography, soil, water and vegetation should be collectively considered in mining management projects to design landscapes that reduce the mining pollution downstream and *within* the reclaimed mines (Nicolau, 2003). The selection of the overburden with the best physical and chemical characteristics in the most superficial layers and the design of a topography that minimizes erosion are important topics to consider in post-mining area management to reduce the metal pollution in constructed ponds and their associated aquatic and terrestrial ecosystems.

CHAPTER 2

Macroinvertebrate biodiversity patterns during primary succession in manmade ponds

Leticia Miguel-Chinchilla[1*], Dani Boix[2], Stephanie Gascón[2] and Francisco A. Comín[1]

Abstract The main aim of this work was to evaluate the primary succession of manmade ponds by studying the temporal patterns of the pond biodiversity parameters. We surveyed the macroinvertebrate community, the water and sediment of 19 manmade ponds of different ages (from 1 to 22 years) located at reclaimed opencast coal mines in northeastern Spain. This study showed an increase of biodiversity with pond age: the oldest ponds showed higher complexity and more rare taxa than the youngest ponds, while the taxonomic richness did not change. These results highlighted the need for using a wide range of biodiversity parameters. Moreover, our results suggest that environmental characteristics of post-mining landscapes are more determinant for the evolving macroinvertebrate community than time because pond age explained less of the biodiversity variance than the environmental characteristics. The changes found in water and sediment during time were not reflected in changes in biodiversity, and the levels of biodiversity in our study area were lower than those of restored or manmade ponds of similar ages.

Key words pond-age, environmental characteristics, taxonomic distinctness, rarity, richness, chronosequence approach, community parameters, opencast coal mines.

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INTRODUCTION

Long-term datasets are necessary to interpret and understand many ecological processes, such as succession. Succession (defined here as the process of change in species composition in an ecosystem over time) is a key process related to the functioning of the ecosystem (Margalef, 1968; Odum, 1969; Gutiérrez & Fey, 1980). However, knowledge about the basic processes driving primary succession in wetlands remains limited (Noon, 1996), particularly with respect to changes in the faunal community (Batzer et al., 2006). Moreover, most successional studies have been based on temporary wetlands and therefore strictly analyzed secondary succession processes (Lake et al., 1989; Boix et al., 2004).

Manmade ponds represent an excellent system for analyzing the primary succession process because they are created on surfaces where an aquatic community has not previously existed and the pond age is usually known (Velasco et al., 1993; Flory & Milner, 2000; Matthews et al., 2009). In addition, biodiversity is a suitable community parameter for studying primary succession because it is important in maintaining the biogeochemical cycles and functioning of the ecosystem (Loreau et al., 2001; Hooper et al., 2005). However, empirical studies describing how biodiversity changes over time in manmade ponds are scarce and most of these studies have been based on taxonomic richness as a biodiversity surrogate. Traditional ecological papers hypothesized that there is an increase in biomass and biodiversity during succession (Odum, 1969; Gutiérrez & Fey, 1980; Legendre et al., 1985). Some authors verified an increase in richness during the initial colonization of newly created ponds (Barnes, 1983; Ruhí et al., 2009, 2012; Marchetti et al., 2010). However, after the initial changes in biodiversity, the rate of acquisition of new species tends to decline within a few years of the onset of succession (Barnes, 1983; Fairchild et al., 1999; Proctor & Grigg, 2006; Marchetti et al., 2010), indicating an equilibrium in the number of species, as predicted by the theory of island biogeography (MacArthur & Wilson, 1967; Whittaker & Fernández-Palacios, 2007). Moreover, over longer time scales, unimodal responses may also be present (Rosenzweig, 1992; Hansson et al., 2005), while other authors found no pattern in long-term studies regarding biodiversity when using taxonomic richness (Gee & Smith, 1995; Spieles et al., 2006).

Nevertheless, no single index alone is a suitable surrogate to represent overall biodiversity (Warwick & Clarke, 1995; Wilsey et al., 2005; Heino et al., 2007). Taxonomic richness alone may not represent all of the biodiversity aspects of the community and therefore has been over used as a measure of biodiversity (Bilton et al., 2006; Gallardo et al., 2011). Therefore, other parameters, such as taxonomic distinctness and rarity, that take into account complementary aspects of the concept of biodiversity should be considered. These two parameters illustrate different but complementary techniques of inferring community characteristics (Heino et al., 2007; Gascón et al., 2009). For example, it has been suggested that pioneering taxa are taxonomically highly related, and lower values of taxonomic distinctness are expected at the initial phases of succession (Ruhí et al., 2009). Moreover, it has been reported that as succession progresses, colonization is mainly driven by the erratic arrival of dispersers with lower dispersal abilities (Ruhí et al., 2013). Therefore, at later successional phases, an increase in the rarity values of macroinvertebrate assemblages is expected due to the erratic arrivals of such taxa that also may produce increased taxonomic unevenness. Accordingly, we expected that the biodiversity of the macroinvertebrate community changes during primary succession.

Thus, our main objective was to analyze the temporal patterns of biodiversity during primary succession using complementary biodiversity parameters. We considered several questions: (1) Is biodiversity changing over time? (2) Are temporal changes in biodiversity related to environmental changes? (3) Is age the main factor explaining the variation in biodiversity? To answer these questions, we studied the biodiversity of the macroinvertebrate community and the environmental characteristics (water, sediment and landscape) of a set of manmade ponds constructed at different times during opencast coal mining reclamation. Due to the particular characteristics of the study site, we included a fourth question: (4) Is the biodiversity of manmade ponds constructed in post-mining landscapes similar to the biodiversity of manmade ponds constructed in other environmental conditions?

METHODS

Sampling and sample processing

In this study we used the macroinvertebrate data and water sediment and landscape characteristics of the 19 manmade ponds located in the reclaimed mines.

In water we considered the following parameters: dissolved oxygen, temperature, conductivity, pH, chlorophyll-*a,* alkalinity, total suspended solids, total suspended organic matter, total dissolved nitrogen, non-purgeable organic carbon, total dissolved phosphorus and 10 dissolved trace metals: Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn. In sediment we considered: pH, conductivity, particle-size (sand, thick silt, thin silt and clay), total carbon, total inorganic carbon, total organic carbon, total sulfur, total nitrogen, total phosphorus and 10 total heavy metals (the same ones analyzed in water). In the landscape we used: pond size, pond littoral slopes, pond basin slope, vegetated pond littoral area, nearest pond distance, nearest river distance and the numbers of ponds and number of rivers in a 1000-m buffer.

Sample collection and processing and the variables used in this chapter were described in the general methods section. Moreover, a summary of the environmental variables used in the statistical analysis is shown in the appendix of this study.

Data analysis

Biodiversity parameters

Because the macroinvertebrate sampling effort was not comparable among ponds, to characterize the macroinvertebrate community, we calculated four biodiversity parameters that did not require abundance estimations: rarefied richness, average taxonomic distinctness, variance of taxonomic distinctness and index of faunal originality as the rarity index. Biodiversity parameters were calculated for each pond in each season.

Species richness increases with sample size, and differences in richness may be caused by sampling differences (Oksanen et al., 2009). Rarefaction is a method for comparing species richness between treatments, after standardization, to account for sampling effort.

Therefore, rarefied richness (RR) minimizes the differences between the sampling effort, collection conditions and organism abundances (Gotelli & Colwell, 2001). In this case, RR was standardized according to the minimum number of invertebrate taxa collected in one sample using the "rarefy" function that was available in the "Vegan" package (Oksanen et al., 2009), which is a statistical package that provides tools for descriptive community ecology developed for the R statistical software (R Core Team, 2012).

Taxonomic distinctness parameters consider the relatedness of species in each sample incorporating the phylogenetic relationships among taxa (Clarke & Warwick, 1998). We considered two taxonomic distinctness parameters, 'Average taxonomic distinctness' (AvTD) and 'Variance in taxonomic distinctness' (VarTD) because there are not sensitive to variation in sampling effort and are calculated with presence-absence data (Clarke & Warwick, 1998). AvTD is the mean path length between any two randomly chosen taxa traced through a Linnaean or phylogenetic classification of the full set of documented taxa (Clarke & Warwick, 1998) and is a proxy of the taxonomic relatedness of the taxa encountered in the analyzed assemblage. In comparison, VarTD is the variance of these pairwise path lengths and reflects the unevenness of the taxonomic tree (Clarke & Warwick, 2001). AvTD and VarTD were calculated with PRIMER v6 using a setting of 100, which is the longest path length in taxonomy. The path lengths between the different taxonomic levels of the classification tree (based on standard Linnaean hierarchical classification) were considered equal. Six taxonomic levels (genus, family, order, class, phylum and kingdom) were considered in the aggregation file.

Finally, we calculated the rarity of the macroinvertebrate community through the Index of Faunal Originality (IFO), which only needs presence/absence data and is, therefore, independent of the taxonomic abundance. IFO was calculated for each manmade pond according to (Puchalski, 1987):

$$IFO = \frac{\sum_{i=1}^{S} \left(1/M_i\right)}{S},$$

where M is the total number of samples in which taxon i occurs (total number of manmade ponds in which a particular taxon appears) and S is the total number of taxa in the corresponding sample (the total number of taxa in the manmade pond for which the index is calculated). The theoretical maximal value of the index is 1, indicating that none of the taxa found in one pond were recorded in another pond.

Statistical analysis

The ponds were grouped into four age categories (Pond Age Categories, hereafter PAC) for statistical analysis: PAC1, 1–5 years (5 ponds); PAC2, 6–10 years (5 ponds); PAC3, 11–15 years (4 ponds); and PAC4, 16-22 years (5 ponds).

To explore biodiversity changes across the PACs, we used linear mixed models (LMM), with PAC as the fixed effect and season as a random effect. The inclusion of season in the random part of the model allowed us to control pseudo-replication problems due to sampling each pond in two different seasons. We performed the linear mixed models using the 'lme' function integrated in the 'nlme' package (Pinheiro et al., 2012) designed for R statistical software (R Core Team, 2012). A ln-transformation of IFO was needed to improve the error fitness to a normal distribution.

The biodiversity levels of the manmade ponds constructed in the reclaimed opencast coal mines were compared with data from other studies undertaken with manmade or restored wetlands and ponds of different ages. We selected nine studies: Barnes (1983), Hov & Walseng (2003), Solimini et al. (2003), Lancaster et al. (2004), Proctor & Grigg (2006), Spieles et al. (2006), Ruhí et al (2009, 2012) and Marchetti et al. (2010) that considered some of the biodiversity parameters used in this study (RR, AvTD, VarTD and IFO) or offered taxonomic lists that allowed their calculation. Note than in Ruhí et al. (2009), we only considered the data for permanent manmade ponds.

To determine whether the environmental characteristics of the manmade ponds vary over time, we used two discriminant analyses (DA), one for water (water-DA) and another for sediment (sediment-DA) characteristics. DA is a multivariate method that generates a series of discriminant functions based on linear combinations of predictor variables that provide the best possible discrimination (or maximal separation) between pre-established groups (Hair et al., 2005; Corstanje et al., 2009). Therefore, DA allows for the statistical determination of significant differences in water and sediment characteristics among the four PACs and thus determines whether environmental characteristics change significantly over time. These analyses were carried out using SPSS 19 for Windows (SPSS Inc., Chicago, IL, USA). Before the analysis, all variables, except pH, were log(x+1) transformed. To reduce multi-collinearity problems in the DA, we excluded from the analysis those highly associated parameters (of water and sediment datasets) based on Spearman rank correlations (if $p < 0.01$ and $r_s \geq 0.7$; Myers, 1986). In such cases only one of the parameters was retained.

Finally, we performed variation partitioning analyses to quantify the proportion of biodiversity variability explained by the four groups of predictors considered in this study: PAC, water, sediment and landscape datasets. This analysis breaks down and quantifies the explained variation in the dependent variables (biodiversity parameters) as pure (or unique) and shared (or joint) effects of a set of predictors (age and environmental datasets). Thus, we distinguished three type of effects: pure (the variation explained by only one dataset without considering the effects the other datasets included in the analysis), shared (the variation explained by one dataset and its interaction with other datasets included in the analysis) and global (the variation explained by one dataset and its interaction with all the datasets included in the analysis). For more information about variation partitioning see Borcard et al. (1992), Heikkinen et al. (2005), Peres-Neto et al. (2006) and Wang et al. (2011). The variation partitioning analyses is integrated in the 'varpart' function as part of the 'Vegan' package (Oksanen et al., 2009) available for the R statistical software (R Core Team, 2012). One variance partitioning analysis was performed with each biodiversity parameter: RR, AvTD, VarTD and IFO. Before performing variation partitioning, we identified the predictor variables within each dataset (water, sediment and landscape) having independent impacts on the biodiversity parameter to only include the environmental variables that significantly explain the variability of each parameter in each analysis. We identified these variables by the use of the 'rand.hp' function, which is a randomization routine integrated into the 'hier.part' package (MacNally & Walsh, 2004) available for the R statistical software (R Core Team, 2012) and which focuses on the analysis of the variance partition of a multivariate data set. This analysis showed the independent contribution toward the explained variance in a multivariate dataset. The results of 'rand.hp' were expressed as Z-scores and the statistical significance was based on the upper 95% confidence limits (Z ≥1.65; MacNally, 2002).

RESULTS

Biodiversity changes across time

Although RR did not show any significant response through succession (i.e., PAC), the other biodiversity parameters did. AvTD, VarTD and IFO significantly increased with pond age (Fig. 5), indicating that as ponds mature, the macroinvertebrate assemblages that inhabit them increase in rarity (IFO), average taxonomic distinctness (AvTD) and unevenness of the taxonomic tree (VarTD).

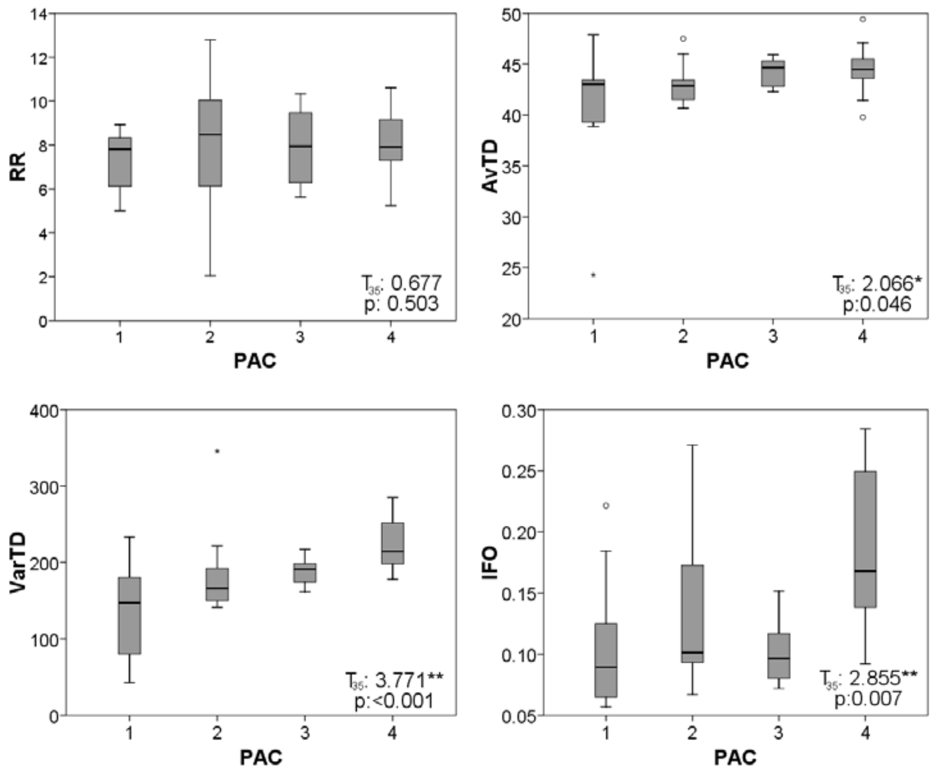

Figure 5: Long-term biodiversity trends. The values of each biodiversity parameter (RR: rarefied richness; AvTD: average taxonomic distinctness; VarTD: variation in taxonomic distinctness; IFO: index of faunal origin) are represented by the pond age category (PAC). Spearman correlations and p-values are shown.

Water and sediment characteristics among pond age categories

The variables included in the water-DA selected after determining the correlations were chlorophyll-a concentration, pH, conductivity, alkalinity, non-purgeable organic carbon, total dissolved nitrogen, total dissolved phosphorus, Al, As, Cr, Cu and Ni. The variables used in the sediment-DA were pH, total inorganic carbon, total organic carbon, total sulfur, thin silt fraction, clay fraction, As, Cr, Cu, Fe, Mn, Ni and Zn.

The discriminant analysis of the water characteristics showed that only the first of the three discriminant functions (DF) was significant in differentiating PACs based on the χ^2 test (p-value < 0.05). The first DF showed a high canonical correlation with PAC (ϱ = 0.827) and accounted for 67.2% of the explained variance. The first three DFs were significant for sediment characteristics (p-value < 0.05). All three had a significantly high canonical correlation coefficient with PAC (ϱ = 0.916, 0.834 and 0.801), accounting for 56.2% of the explained variance for the first DF to 19.2% for the third DF. Both water-DA and sediment-DA clearly discriminated younger ponds (PAC1) from the rest of the ponds (Fig. 6A & B).

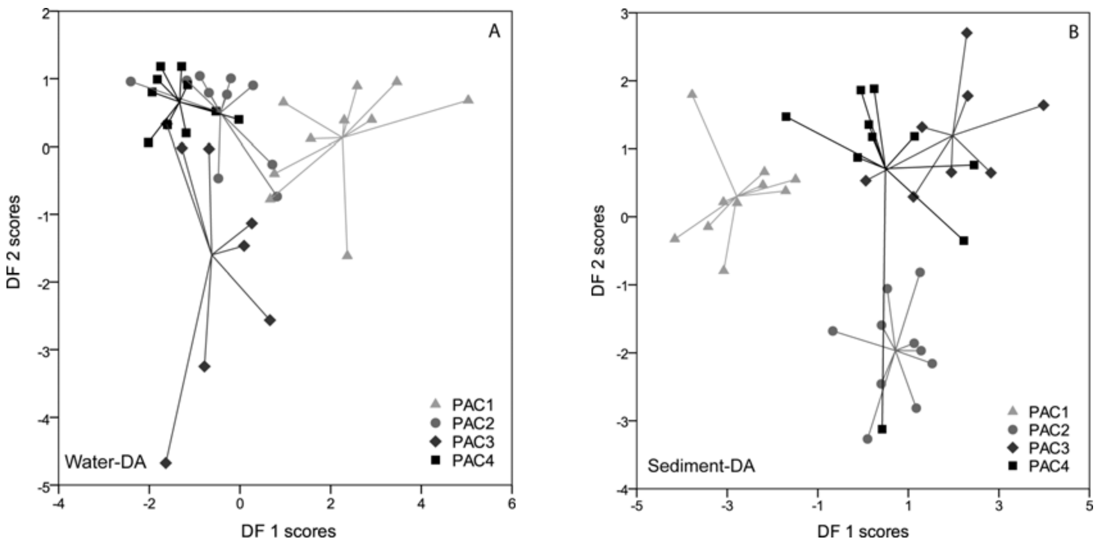

Figure 6: Scatter plot of the first two functions (DF1 and DF2) of the discriminant analysis using water (A) and sediment (B) characteristics to differentiate pond age categories (PAC).

The differences in water characteristics (Fig. 6A) were related to total nitrogen content, which was the variable better correlated to the first DF (r = 0.453), indicating that youngest ponds had higher nitrogen concentrations.

The first DF obtained with the sediment dataset (Fig. 6B) was positively correlated with total organic carbon (r = 0.361), total sulfur (r = 0.221) and several heavy metals (iron r = 0.250; arsenic r = 0.244; and chromium r = 0.208), while the pH (r = -0.286) and total manganese content (r = -0.236) were negatively correlated. Thus, youngest ponds had less total organic carbon and metal content than older ponds. Both DAs indicated that the youngest ponds (PAC1) were different in their environmental characteristics compared to the other three PACs. Consequently, the main environmental changes were detected when comparing youngest ponds (from 0 to 5 years old) with older ponds (from 6 to 22 years old), in which environmental conditions remained similar (Fig. 6 A & B).

Factors explaining the biodiversity variability

The variation partitioning results (Table 2), performed after variable selection (see Table 3 to identify selected variables after the randomization routine) showed that the proportion of variation explained by pond age, water, sediment and landscape datasets varied with respect to the biodiversity parameter analyzed.

Table 2: Results of the variation partitioning analysis for the biodiversity parameters (RR: rarefied richness; AvTD: average taxonomic distinctness; VarTD: variation in taxonomic distinctness; IFO: index of faunal origin) with respect to water, sediment, landscape and pond age categories (PAC). The proportions of unexplained, pure, global and shared variations are shown for pond age and the other three sets of variables. The significance of pure and global effects was tested (*0.05, **0.01). Significance tests for the combined effects are not available.

		RR	AvTD	VarTD	IFO
Water	Pure	0.12*	0.17	0.20**	0.10
	Global	0.31**	0.51	0.62**	0.20*
Sediment	Pure	-	-0.01	0.04	0.13
	Global	-	0.15*	0.42**	0.29*
Landscape	Pure	0.04	0.03	0.01	-
	Global	0.27**	0.33**	0.39**	-
PAC	Pure	0.00	0.00	0.04	-0.02
	Global	-0.01	0.07	0.27**	0.14**
PAC & Water	Shared	-0.03	0.07	0.20	0.09
PAC & Sediment	Shared	-	0.08	0.23	0.16
PAC & Landscape	Shared	0.00	0.07	0.24	-
Unexplained		0.63	0.47	0.26	0.62

Overall, the environmental characteristics and pond age explained a higher proportion of the variation in the taxonomic distinctness parameters (more than 50%) than in RR and IFO (less than 40%). This indicates that the age and the environmental characteristics of the ponds contributed significantly, but to a lesser extent, to the observed variation in taxonomic richness and abundance of rare taxa. Our results showed that the observed variability in biodiversity parameters was not purely explained by pond age. When also accounting for the shared variability of pond age with the other environmental variables (i.e., the global pond age effect), pond age significantly explained VarTD and IFO variability (Table 2). Water (pure and global) significantly explained some of the variability in RR and VarTD and also explained some of the variability in IFO, when considered in relation to the other data sets (global effect). Sediment was significant for AvTD, VarTD and IFO only with respect to the global effect and, similarly, landscape only had significant global effects in explaining the variability of RR, AvTD and VarTD. In general, the proportion of variation explained by environmental characteristics was higher than the variation explained by pond age for any of the biodiversity parameters tested, indicating a small effect of pond age on biodiversity.

DISCUSSION

Curiously, taxonomic richness – the most widely used index of biodiversity – was the only parameter that did not show any significant response when comparing pond age categories. Other studies performed in constructed (Gee et al., 1997) or restored wetlands (Marchetti et al., 2010) of similar life spans (from 0 to 20/25 years in age) also failed to detect a long-term increase in taxonomic richness, although Marchetti et al. (2010) detected an increase in richness during the first five years after pond restoration.

This result does not necessarily mean that community composition does not change. Spieles et al. (2006) found a change in guild dominance across time, although basic community parameters (richness and abundance) showed no significant differences over a 10-year range. Similarly, the number of taxa in our study did not significantly change, whereas the taxonomic structure of the assemblages and rarity did (Fig. 5). Colonization processes in newly created ponds have been suggested to be driven by a few taxonomic groups, and during succession, new taxa arrive, while some pioneering taxa disappear (Layton, 1991; Ruhí et al., 2009). In addition, an increase in the arrival of passive dispersers or active dispersers having lower dispersal abilities over time has been described (Ruhí et al., 2013). Thus, a balance between the arrival and disappearance of taxa may explain the lack of richness differences among pond age categories. Moreover, the increase in AvTD and VarTD suggests that the new taxa arriving in the manmade ponds belong to distant taxonomic groups (because we detected an increase the phylogenetic distance among taxa, AvTD) and were unequally distributed in the taxonomic tree (because of the increase of VarTD). The significant increase in rare taxa (IFO values) could be due to the erratic arrival of new taxa and it is reasonable to think this also may contribute to the increase in VarTD values.

Table 3: Variables selected from the three groups of explanatory variables (water, sediment and landscape) for each biodiversity parameter (RR: rarefied richness; AvTD: average taxonomic distinctness; VarTD: variation in taxonomic distinctness; IFO: index of faunal origin) introduced in the variation partitioning analyses.

	RR	**AVTD**	**VARTD**	**IFO**
Water	Chlorophyll-a	pH	Chlorophyll-a	Chlorophyll-a
	Suspended organic matter	Non purgueable organic carbon	Non purgueable organic carbon	Dissolved Oxygen
	Total suspended solids	Total dissolved nitrogen	Total dissolved nitrogen	
		Al	Ni	
		Fe	Fe	
		Zn	Zn	
Sediment		Total carbon	Total carbon	Total carbon
		Clay fraction	Clay fraction	Total sulfur
			Conductivity	Total organic carbon
			As	Total nitrogen
				As
				Cr
				Ni
Landscape	Littoral vegetation area	Littoral vegetation area	Littoral vegetation area	
		Pond size	Pond size	
			Numbers of ponds in a 1000-m buffer	

Despite the positive tendency detected in AvTD, VarTD and IFO over time, we noticed that PAC alone did not explain a great proportion of the biodiversity. This means that changes that occur over time were not the main source of biodiversity variability in the macroinvertebrate community of the manmade ponds. Indeed, we found a higher importance of environmental characteristics over pond age, explaining the biodiversity variability. However, when differences in water and sediment among PAC were found (the environmental characteristics of PAC1 were different from PAC 2 to 4), we did not detect clear differences in biodiversity parameters (see Figs 5 and 6). The lack of synchronization in the changes in pond characteristics and biodiversity, combined with the significant contribution of the landscape features to the explanation of biodiversity variability (i.e., taxonomic distinctness), suggested that local environmental characteristics may play an important role in the evolving macroinvertebrate community.

The biodiversity of the manmade ponds constructed in reclaimed opencast coal mines was low compared to other constructed or restored ponds of similar pond ages (Table 7). Indeed, the biodiversity in our study showed lower values than other manmade ponds located in post-mining landscapes, as was the case in Moura (in Australia). The low biodiversity values combined with the greater environmental than PAC explanation of the biodiversity variability among ponds suggested that the macroinvertebrate community inhabiting the ponds constructed in the reclaimed coal mines of Teruel were constrained by the particular characteristics of the study area. The homogeneity of the pond habitat (e.g., littoral ponds were dominated by *Typha* sp. and ponds had similar sediment granulometry) may be one important reason for the low biodiversity values because faunal biodiversity is positively correlated with the complexity of the pond habitat (O'Connor, 1991; Pedruski & Arnott, 2011). Moreover, it is possible that the manmade ponds were polluted due to coal mining despite mine reclamation. In fact, several metals had a relevant contribution to the biodiversity explanation (Table 3), and may contribute to the low biodiversity values due their negative effects over macroinvertebrate community (Clements, 1994; Van Damme et al., 2008; Iwasaki et al., 2009). Finally, the development of the macroinvertebrate community may have been limited by the recruitment of organisms (Palmer et al., 1996; Brady et al., 2002; Brederveld et al., 2011). No natural wetlands or ponds were found near the coal mines, and the manmade ponds were isolated from water courses. Therefore, the source of macroinvertebrates was primarily restricted to distant streams. This fact may reduce the probability of colonizing the manmade ponds because the macroinvertebrate

Table 4: Biodiversity data (RR: rarefied richness; AvTD: average taxonomic distinctness; VarTD: variation in taxonomic distinctness; IFO: index of faunal origin) collected from several studies on macroinvertebrate communities in constructed or restored ponds. The mean biodiversity data are presented for time periods that were similar to the age categories used in this study.

Site	Studied community	Purpose	Age	Parameters					Reference
				Richness	RR	AvTD	VarTD	IFO	
Teruel (Spain)	Genus	Runoff contron in reclaimed opencast coal mines	1 to 5	-	7.3	41.09	138.19	0.11	This paper
			6 to 10	-	8.0	43.20	188.12	0.13	
			11 to 15	-	7.9	44.23	188.24	0.10	
			16 to 22	-	8.0	44.44	223.47	0.18	
California (USA)	Genus	Return degrade wetlands to functional natural ecosystems	1 to 5	23.5	21.3	44.85	253.35	0.34	(Marchetti et al. 2010)
			10	29.0	25.4	44.76	233.60	0.38	
			>20	30.0	23.6	44.66	224.30	0.52	
Catalonia (Spain)	Genus	Habitat and species recovery	1 to 3	-	-	59.91	355.85	-	(Ruhí et al. 2009)
Rome (Italy)	Genus & Family	Wastewater aquaculture treatment	1 to 3	26.0	24.5	49.23	268.23	0.44	(Solimini et al. 2003)
Kalmar (Sweden)	Specie	Wastewater airport treatment Increase of biodiversity	1 to 3	22.6	-	66.75	247.13	-	(Ruhí et al. 2012)
			7	27.5	-	65.90	187.96	-	
			13	11.0	-	75.95	181.49	-	
Moura (Australia)	Family	Pit ponds result of coal mining	0 to 4	14.0	9.3	43.02	289.97	0.19	(Proctor and Grigg 2006)
			12 & 14	14.7	7.1	40.79	287.07	0.22	
			17 & 22	14.5	8.3	41.41	294.40	0.20	
Central Ohio (USA)	Genus	Wetland mitigation lost	10	23.5	25.5	48.40	184.08	0.53	(Spieles et al. 2006)
Dunfermline (UK)	Genus	Urban drainage treatment ponds	1 to 5	21.6	-	62.86	512.56	0.48	(Lancaster et al. 2004)
Trogstad (Norway)	Genus	Nutrient pollution treatment	1 to 5	24.2	-	39.77	254.85	0.55	(Hov and Walseng 2003)
Dorset (UK)	Genus	Pit ponds result of clay extraction	1 a 3	29.7	-	45.08	229.63	0.33	(Barnes 1983)
			15	41.0	-	46.71	286.10	0.42	

community of lotic ecosystems is different and shows lower dispersal abilities than the macroinvertebrates inhabiting lentic ecosystems (Ribera & Vogler, 2000; Marten et al., 2006).

Comparing biodiversity values with other studies allow to search for temporal biodiversity patterns among different regions. To perform this comparison, we selected AvTD and VarTD because of their lack of dependence on sampling effort allow for a comparison across studies from different localities or from regions using different sampling methods (Clarke & Warwick, 1998). The AvTD vs. VarTD scatter plot (Fig. 7) showed no relationship between biodiversity and pond age when the manmade wetlands and ponds of different study sites were compared. Moreover, neither the geographic area nor the construction objective were determining factors (see Table 4); therefore, the local conditions of each study appear to be more important for explaining macroinvertebrate biodiversity than the general patterns.

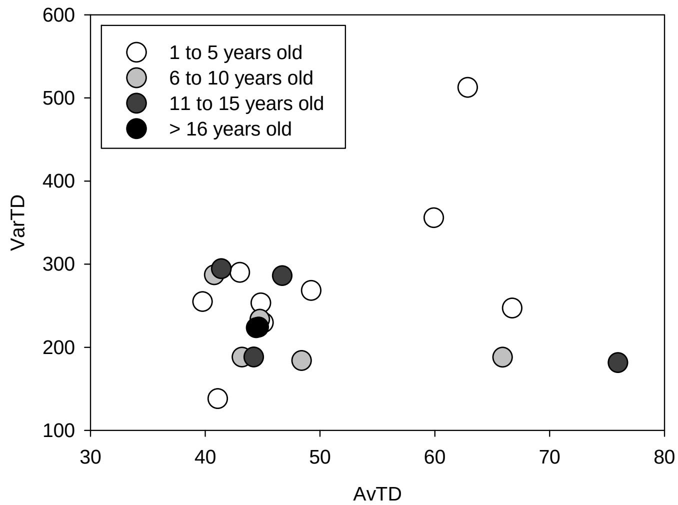

Figure 7: Scatter plot of variation in taxonomic distinctness (VarTD) against average taxonomic distinctness (AvTD) to compare the biodiversity of 10 different study sites in relation to pond age. Data corresponding to table 4 were used.

CONCLUSIONS

In summary, this study demonstrated that even constructed ponds located in post-mining landscapes and without an enhanced biodiversity purpose showed an increase in almost all of the studied biodiversity parameters over time. The only biodiversity parameter that did not increase is the parameter that is mainly used to assess biodiversity (i.e., richness). This fact highlights the importance of using a range of biodiversity parameters to study the macroinvertebrate community, which agrees with previous studies (Wilsey et al., 2005; Heino et al., 2007; Gascón et al., 2009; Gallardo et al., 2011). Our results suggest that environmental factors better explain the configuration of pond biodiversity than pond age. This fact, coupled with the low biodiversity detected in our study area, suggest that environmental conditions may restrict the number and type of taxa that are able to colonize and become established in manmade ponds and therefore restrict the evolution of the macroinvertebrate community. However, because biodiversity parameters increased over time (except for rarefied richness) and natural ponds are absent from the studied area, manmade ponds constructed during reclamation activities to control runoff provide both biological and landscape diversity, at least at a regional scale. Due to the particularities derived from the location of the manmade ponds in post-mining landscapes, care should be taken when extrapolating biodiversity findings from this study to other settings.

Appendix Chapter 1: Summary of environmental characteristics

Summary of the environmental characteristics used in this study. Mean and standard errors are shown by pond age categories.

	Pond Age Categories							
	1		2		3		4	
Water	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Chlorophyll-a (μg l^{-1})	1.92	0.38	12.92	9.27	2.57	0.71	3.53	0.83
Dissolved oxygen (mg l^{-1})	10.89	0.87	7.98	0.93	9.5	1.01	7.04	0.41
pH	7.83	0.37	7.86	0.14	7.45	0.4	7.59	0.12
Conductivity (μS cm^{-1})	1714	377	1227	153	1555	139	1183	136
Suspended organic matter (mg l^{-1})	2.84	0.22	4.3	1.08	2.4	0.21	3.54	0.57
Total suspended solids (mg l^{-1})	7.14	0.99	8.73	2.47	4.92	0.86	10.55	1.53
Alkalinity (mg l^{-1})	43.45	7.34	104.67	17.07	46.96	17.81	144.62	24.88
Non purgeable organic carbon (mg l^{-1})	4.84	1.01	6.91	0.79	4.24	0.88	5	0.8
Total dissolved nitrogen (mg l^{-1})	1.92	0.74	0.58	0.08	0.33	0.07	0.44	0.08
Total dissolved phosphorus (mg l^{-1})	0.0025	0.0013	0.0025	0.0008	0.0015	0.0005	0.0009	0.0003
Al (mg l^{-1})	0.102	0.0101	0.1133	0.0043	1.0343	0.7587	0.118	0.0014
As (mg l^{-1}	0.0006	0.0003	0.0007	0.0005	0.0006	0.0005	0.0022	0.002
Cr (mg l^{-1})	0.0001	0.0001	<0.0001	<0.0001	0.0005	0.0004	0.0001	0.0001
Cu (mg l^{-1})	0.0624	0.0554	0.0623	0.0278	0.047	0.0257	0.0948	0.0679
Fe (mg l^{-1})	0.0006	0.0002	0.0002	0.0001	0.0005	0.0001	0.0002	0.0001
Ni (mg l^{-1})	0.0722	0.0227	0.0555	0.0152	0.0553	0.0248	0.0456	0.013
Zn (mg l^{-1})	0.0076	0.0052	ND	-	0.0129	0.0098	0.0038	0.0025
Sediment								
pH	7.78	0.09	7.73	0.06	6.17	0.6	7.65	0.07
Conductivity (μS cm^{-1})	1261	583	829	115	1368	43	1351	231
Total carbon (%)	1.33	0.34	2.82	0.54	2.8	0.66	5.4	0.66
Total inorganic carbon (%)	0.68	0.34	0.96	0.26	0.81	0.41	2.1	0.28
Total organic carbon (%)	0.66	0.1	1.86	0.44	1.99	0.36	3.3	0.67
Total sulfur (%)	0.12	0.04	0.14	0.07	0.47	0.12	0.67	0.16
Total nitrogen (%)	0.04	0.01	0.08	0.01	0.12	0.02	0.15	0.05
Total phosphorus (mg/kg^{-1})	306.78	18.02	327.48	43.41	388.66	56.55	403.39	30.55
Thin silt fraction (%)	30.98	6.15	38.17	1.93	44.73	6.89	19.9	3.21
Clay fraction (%)	5.69	1.11	7.74	0.65	9.72	1.61	4.69	1.01
As (mg kg^{-1})	11.61	2.04	16.24	2.4	16.46	1.56	21.81	3.09
Cr (mg kg^{-1})	20.46	2.01	31.24	2.36	30.99	2.53	21.02	2.67
Cu (mg kg^{-1})	5.11	1.38	9.47	5.37	16.45	3.04	4.77	1.57
Fe (mg kg^{-1})	20449.21	2034.06	31998.76	1979.48	34135.83	2333.75	32767.52	6956.7
Mn (mg kg^{-1})	902.97	343.66	270.54	37.89	274.52	64.18	464.63	111.43
Ni (mg kg^{-1})	137.75	32.03	85.86	33.52	220.26	56.1	63.96	23.16
Zn (mg kg^{-1})	56.21	12.91	41.6	4.72	95.37	17.97	235.84	119.45
Landscape								
Pond size (m^2)	59646	39334	10304	6933	20520	9158	5531	1027
Littoral vegetation (m^2)	4763	2298	3328	2068	10113	5123	1961	992
Number of ponds in a 1000-m buffer	2.2	0.2	3.2	0.58	2.25	0.48	2.6	0.4

CHAPTER 3

Taxonomic and functional successional patterns in poorly and easily dispersing macroinvertebrates: a case study from isolated manmade ponds at reclaimed opencast coal mines

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Abstract It has been proposed that communities change from *r* to *K* strategies during primary succession. However, because easily dispersing organisms are expected to arrive first in newly created habitats and they show trait characteristics associated more often with *K* strategies, we hypothesized that the *r* to K trajectories would be more closely followed by poorly dispersing organisms. Moreover, we expected that macroinvertebrate communities would converge in their functional composition due to deterministic forces while diverging as taxonomic assemblages due to historical contingent forces (stochastic drift and biotic interactions). However, we also expected that dispersal abilities of the organisms may affect these tendencies. To address these questions, macroinvertebrates were sampled from isolated manmade ponds of different ages (1 to 22 years old) constructed at reclaimed opencast coal mines. In accordance with our expectations, only poorly dispersing organisms exhibited a slight shift from *r* to *K* strategies, the community diverged taxonomically along the primary succession gradient, and historical contingent forces showed greater effects on easily dispersing organisms. In contrast, the community did not converge in its functional composition. The weak differences observed among the macroinvertebrates from ponds of different ages suggested that limiting environmental conditions prevented the organisms from evolving to a more structured community.

Key words primary succession, dispersal, biological traits, taxonomic composition, deterministic forces, stochasticity

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INTRODUCTION

Successional dynamics are a key conceptual process in community ecology (Margalef, 1968; Odum, 1969; Gutiérrez & Fey, 1980), but surprisingly, information concerning the process of primary succession in communities of aquatic macroinvertebrates is still scarce (Flory & Milner, 2000; Cañedo-Argüelles & Rieradevall, 2011). Newly created habitats (i.e., manmade ponds) have proven to be ideal systems for testing temporal community dynamics (Noon, 1996; Chase, 2007; Hassall et al., 2012; Ruhí et al., 2013), and may be of especial relevance for analyzing primary succession. However, the monitoring of these new ecosystems usually did not reach 5 years in duration, and most succession research has been based on the study of temporary wetlands, so that secondary successional processes were analyzed (e.g., Lake et al., 1989; Velasco et al., 1993; Boix et al., 2004).

Traditionally, it has been proposed that, if an ecological vacuum is filled (e.g., construction of a manmade pond), selection will shift a population from *r* toward the endpoint *K*, from resilience to more competitive taxa (MacArthur & Wilson, 1967; Townsend & Hildrew, 1994). At the initial stages of succession, organisms of smaller sizes, with shorter life cycles, and good dispersal abilities are expected to be more abundant, while at advanced stages of succession, increases in the organisms' body sizes, life cycle durations, and the incidence of passive dispersal would be expected. Small-bodied organisms often correlate with short life cycles (Southwood, 1977; Wiggins et al., 1980; Townsend & Hildrew, 1994; Bêche et al., 2006), a high tolerance to adverse conditions (Townsend & Hildrew, 1994; Townsend et al., 1997; Verberk et al., 2008) and the occupation of lower trophic positions (Woodward & Hildrew, 2002; Brown et al., 2004; Jonsson et al., 2005). In freshwater invertebrates, active dispersal results predominantly from the flight of adult insects, which show different functional characteristics related to their particular dispersal abilities (Bilton et al., 2001; Harrison & Dobson, 2008). Where the orders Ephemeroptera, Plecoptera, Trichoptera and Diptera, with poor power of flight, are typically small, short-lived, cryptically colored, non-predatory taxa (i.e., fitting the *r*-strategy), the Coleoptera, Hemiptera and Odonata, with better flight and dispersal abilities, are typically large and brightly colored, live relatively long adult lives and are frequently the top predators in aquatic systems (i.e., fitting more the *K*-strategy). Consequently, we proposed that the expected succession trend along the *r*/*K* theory gradient in the macroinvertebrate community may be better followed by poorly dispersing than by easily dispersing organisms.

Whether the structure of ecological communities is deterministic or historically contingent (from stochastic drift and biotic interactions) has become increasingly controversial (Samuels & Drake, 1997; Belyea & Lancaster, 1999; Chase, 2003; Fukami & Wardle, 2005). The convergence of biological communities has been related to deterministic forces rooted in the climax concept of succession (Clements, 1916). In other words, communities that develop under similar conditions would converge on common structures (Samuels & Drake, 1997; Fukami & Wardle, 2005). The alternative view, the idea of a historically contingent development of communities (Gleason, 1927; Diamond, 1975), suggests that the effects of stochastic drift, which varies the sequence and timing of species' arrivals, can cause divergences in community structure among localities, even under identical environmental conditions and regional species pools (Drake, 1990; Law & Morton, 1993; Fukami & Wardle, 2005). Studies of convergence and divergence implicate a variety of mechanisms and processes spanning different scales, but convergence has usually been detected at coarse structural levels, such as functional perspective, while divergence has been detected at finer levels, such as taxonomic composition (Samuels & Drake, 1997). Thus, using both taxonomic and functional approaches help in studying the mechanisms driving succession processes. Furthermore, dispersal ability has been recognized as a central component to community development during the successional processes (Belyea & Lancaster, 1999; Young et al., 2001; Chase, 2003); therefore, we also expected that deterministic and historical contingency forces had different effects, depending on the dispersal abilities of the organisms.

To the analysis of primary succession, extensive sampling over time is needed to adequately characterize individual ponds (Zedler & Callaway, 1999; Fairchild et al., 2000; Ruhí et al., 2012). However, studies have usually only considered the colonization phase, due to their limited temporal range (Velasco et al., 1993; Ruhí et al., 2009). In an attempt to overcome this problem, an age-series approach (in which communities from a set of differently aged replicated habitats are taken to represent stages in community development from the youngest to the oldest conditions) has frequently been adopted

(Barnes, 1983; Fukami & Wardle, 2005; Bloechl et al., 2010). The present study focused on macroinvertebrate organisms from isolated manmade ponds constructed at different time-periods during reclamation activities at opencast coal mines. Consequently, the studied ponds have different ages (from 1 to approx. 22 years old), were constructed under similar conditions and were affected by similar climatic conditions which made them appropriate for the use of the chronosequence approach for studying primary succession (Majer & Nichols, 1998; Walker et al., 2010). Moreover, the isolated character of the manmade ponds made them excellent places for studying the effects of the dispersal abilities of the organisms.

Specifically, the main objective of this study was to analyze the changes in taxonomic and functional composition along the gradient of primary succession while taking into account the dispersal mode of the organisms. We hypothesized that organisms have different successional patterns depending on their dispersal modes. Thus, (1) the shift from *r* to *K* strategies is expected to poorly dispersing and not to easily dispersing organisms, and (2) we expected that deterministic forces mainly drove functional composition and historical contingent forces mainly drove taxonomic composition but with differences among poorly and easily dispersal organisms.

METHODS

Sampling and sample processing

In this study we used the macroinvertebrate data of the 19 manmade ponds located in the reclaimed mines.

Sample collection and processing of macroinvertebrate community were described in the general methods section.

Biological traits

To study the role of dispersal, we separately analyzed two fractions of the community, the poorly and easily dispersing organisms, using the genus-trait information according to

Tachet et al. (2000). Genus-trait information was structured using a fuzzy-coding technique (Chevenet et al., 1994): scores ranged from '0', indicating 'no affinity', to '5', indicating 'high affinity' for a given genus–trait category. Thus, in the poorly dispersing organisms category, taxa with 'aerial active dispersal affinity' lower than 3 were included, whereas in the easily dispersing category, taxa with an 'aerial active dispersal affinity' equal to or higher than 3 were included. To perform the functional characterization of the macroinvertebrate community, we selected 3 biological traits from Tachet et al. (2000): body size (with 7 categories: < 0.25, 0.25-0.5, 0.5-1, 1-2, 2-4, 4-8, > 8 cm), life cycle duration (with 2 categories: ≤ 1 year, > 1 year), and feeding habits (with 8 categories: absorber, deposit feeder, shredder, scraper, filter-feeder, piercer, predator, and parasite). We divided the piercer category into animal-piercers and plant-piercers according to their diet so finally 9 categories were considered in the feeding habit trait.

Data analysis

We integrated spring and summer data in the same matrix to perform the data analysis. Presence/absence data were used to remove the effects of abundance and give equal weight to taxa that were less common (Proctor & Grigg, 2006; Lawrence et al., 2010). Thus, working with presence/absence data allows for the detection of changes in the macroinvertebrate assemblages due to the appearance and disappearance of taxa instead of changes due to variations in the relative abundance of taxa. To obtain the proportion of a given trait category found in the invertebrate communities from each pond, the affinity scores were weighted by the presence/absence of each genus, and these values were summed for any given trait category. To remove the effects of taxonomic richness among the ponds, the resulting trait categories were rescaled to sum up to one for each trait in each pond (Gayraud et al., 2003).

Moreover, we grouped the ponds into four categories according to their ages (Pond Age Categories, hereafter PAC) to perform the statistical analysis: PAC1, 1-5 years old (5 ponds); PAC2, 6-10 years old (5 ponds); PAC3, 11-15 years old (4 ponds); and PAC4, over 16 years old (5 ponds).

Changes in the taxonomic and functional compositions among the PAC were explored using an analysis of similarity (ANOSIM; Clarke & Green, 1988; Clarke, 1993). We performed the analysis for the easily and poorly dispersing organisms separately. ANOSIM returns a test statistic R, which represents the degree of difference between the sites and a p-value expressed as a percentage. When R is negative or close to 0, similarities within groups (ponds from the same PAC) and among groups (ponds from different PAC) are equivalent. In contrast, differences among the groups exist when the R values approach 1 (Clarke & Warwick, 2001). R-values > 0.5 indicated clear differences between groups with some degree of overlap (Clarke & Gorley, 2006). Taxonomic richness (nº taxa) was calculated for both easily and poorly dispersing organisms. To test for differences among PAC in richness between dispersal abilities (easy and poor), ANCOVA was performed using dispersal ability as the fixed factor, PAC as the covariable, and richness as the response variable. Moreover, to detect differences in dominance among poorly dispersing organisms (proportion of poorly dispersing organisms relative to the total number within each pond) among the PAC we used ANOVA. We only performed this analysis for the poorly dispersing organisms because easy and poor dominance are complementary metrics. Finally, we used the similarity of percentages analysis (SIMPER; Clarke, 1993) to calculate the taxonomic and functional similarities among and within PAC. This analysis was only performed when the ANOSIM detected significant differences among the PAC. SIMPER also showed the relative contributions of individual taxa and trait categories to the similarities among groups (in this case the PAC).

To assess the convergence or divergence of taxonomic and functional composition in the communities, we calculated similarity values between pairs of ponds within each PAC using SIMPER analysis. If there was convergence, the similarity between pairs of ponds within each PAC would increase from PAC1 to PAC4. We calculated these trajectories applying Spearman correlations to the taxonomic and functional matrices.

ANOSIM and SIMPER analyses were computed with PRIMER Version 6.0 software (Clarke & Gorley, 2006). We converted taxonomic and functional data into a resemblance matrix using Bray-Curtis distances. The functional data matrix was previously square-root transformed. ANCOVA and ANOVA were performed using the "aov" function from the "stats" package in R (R Core Team, 2012). Spearman correlations were calculated using SPSS 19 for Windows.

RESULTS

Poorly and easily dispersing organisms among the PAC

Poorly dispersing organisms (40 taxa) were characterized by the Diptera, Heteroptera, Ephemeroptera, Trichoptera, Oligochaeta and Mollusca (Table 5). In contrast, easily dispersing organisms (55 taxa) were primarily composed of Coleoptera, Odonata and Heteroptera, although we also found Ephemeroptera and Diptera at low proportions.

Table 5: Percentage contribution from each taxonomic group to the total number of taxa of poorly and easily dispersing organisms by pond age categories (PAC).

Taxonomic group	Poorly dispersing organisms				Easily dispersing organisms			
	PAC1	PAC2	PAC3	PAC4	PAC1	PAC2	PAC3	PAC4
Coleoptera	0.54	0.00	0.00	0.00	24.22	26.64	20.84	16.76
Diptera	22.30	21.38	22.24	29.75	0.54	5.57	0.80	5.85
Ephemeroptera	7.32	5.86	6.81	10.85	0.67	1.63	1.20	1.06
Heteroptera	10.95	10.36	10.62	5.92	6.08	2.79	6.01	1.09
Odonata	0.00	0.00	0.00	0.00	20.26	19.67	22.44	13.09
Trichoptera	3.22	0.77	3.61	5.50	0.00	0.00	0.00	0.83
Megaloptera	0.00	0.00	0.00	1.84	0.00	0.00	0.00	0.00
Oligochaeta	2.69	3.51	3.81	4.28	0.00	0.00	0.00	0.00
Bivalva	0.00	0.00	0.00	0.55	0.00	0.00	0.00	0.00
Gastropoda	1.21	1.82	1.60	2.64	0.00	0.00	0.00	0.00

ANOSIM performed with taxonomic data showed no significant differences among the PACs, neither for poorly nor for easily dispersing organisms (p-value > 0.05). We did not find any relationship between taxonomic richness and the PACs (ANCOVA; $F_{1,34}$= 1.777, p= 0.286), and no differences were found in the interactions between PAC and dispersal abilities (ANCOVA; $F_{1,34}$= 2.618, p= 0.115), demonstrating that the taxonomic richness of poorly and easily dispersing organisms varied in the same way. Similarly, the dominance of poorly dispersing organisms also showed no significant differences among PACs (ANOVA; $F_{1,17}$= 3.108, p= 0.096).

The poorly dispersing organisms found in the manmade ponds were functionally characterized as having sizes between 0.25 and 2 cm; they exhibited both short (< 1 year) and long (> 1 year) life cycle durations, although the affinity for short life cycles was slightly higher; and the community was composed of deposit feeders, scrapers, shredders, predators and animal-piercers. On the other hand, the easily dispersing organisms exhibited sizes similar to the poorly dispersing organisms (between 0.25-0.5 cm and 1-2 cm); they showed short life cycle durations (the affinity to long life cycles were really scarce); and they exhibited predominantly predator behaviors (including animal-piercing), although they also showed high affinity towards the shredders feeding habits.

The functional similarities among the PACs were generally high for both types of organisms, ranging from 78 to 90% (Table 6). When critically analyzing the results at a functional level, some weak (R < 0.5) but significant differences appeared among the PACs for the poorly as well as for the easily dispersing organisms (Table 6).

Table 6: ANOSIM and SIMPER results performed with functional data. Only significant results are shown.

		ANOSIM		SIMPER
		R	p-value	Similarity percentage
Poorly dispersal	PAC4-PAC1	0.485	0.024	89.97
Easily dispersal	PAC4-PAC1	0.432	0.008	77.51
	PAC3-PAC1	0.375	0.024	86.48
	PAC2-PAC1	0.300	0.048	88.57

Poorly dispersing organisms showed differences between the youngest (PAC1) and oldest ponds (PAC4) while easily dispersing organisms showed differences between the youngest ponds (PAC1) and the other three PACs (PACs 2 to 4). Thus, poorly dispersing organisms that lived in PAC1 showed higher affinities for smaller body sizes (<0.25-0.5 cm), shorter life cycles (< 1 year), and scraper and piercer feeding habits than did PAC4 (SIMPER; Table 7). Easily dispersing organisms that lived in PAC1 showed higher affinities for piercer feeding habits and the largest body sizes (4-8 cm). In contrast, a higher affinity for deposit feeders, scrapers and predators with variable body sizes and life cycle durations was observed in the rest of the PACs (SIMPER; Table 8).

Table 7: SIMPER performed for poorly dispersing organisms. Trait category contribution (percentage) to dissimilarity between the pairs of ponds age categories (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: >16 years) that showed significant differences in ANOSIM (see results section). Trait categories contributing globally up to 90% are shown.

		PAC4 vs. PAC1	
Biological Trait	**Trait category**	**Relation**	**Contribution (%)**
Life cycle duration	>1year	>	12.93
Feeding habits	Animal-piercer	<	11.79
Body size	0.25-0.5cm	<	9.56
Feeding habits	Filter-feeder	>	7.48
Body size	2-4cm	>	6.39
Feeding habits	Herbivorous-piercer	<	6.37
Body size	0.5-1cm	>	6.22
Body size	4-8cm	>	5.83
Feeding habits	Shredder	>	5.66
Feeding habits	Predator	>	5.34
Body size	≤0.25cm	<	4.48
Body size	1-2cm	=	3.57
Life cycle duration	≤1year	<	3.54
Feeding habits	Scraper	<	3.08

Taxonomic and functional convergence or divergence

The SIMPER analysis performed by pairs of ponds within each PAC, and the Spearman correlation showed that taxonomic composition for both poorly and easily dispersing organisms diverged (decreases of similarity within PACs from PAC1 to PAC4; Figs. 8 A and B). However, no convergence patterns were observed for the functional approaches. Instead, the functional characteristics of easily dispersing organisms diverged (Fig. 9A) and poorly dispersing organisms maintained high similarity in the four PACs (Fig. 9B).

DISCUSSION

Shift from *r* to *K* strategies

We did not find taxonomic differences among PACs for either easily or poorly dispersing organisms. Similarly, taxonomic richness and dominance by easily dispersing organisms did not show any significant differences among PACs. At the functional level, and although the similarity among PACs was quite high, some significant but small differences arose. Thus, according to our hypothesis, the results suggested a slight trend from *r* to *K* strategies for poorly dispersing organisms along a successional gradient. Specifically, the

Table 8: SIMPER performed for easily dispersing organisms. Trait category contribution (percentage) to dissimilarity between the pairs of ponds age categories (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: >16 years) that showed significant differences in ANOSIM (see results section). Trait categories contributing globally up to 90% are shown.

PAC4 vs. PAC1			PAC3 vs. PAC1			PAC2 vs. PAC1		
Trait category	**Relation**	**Contribution (%)**	**Trait category**	**Relation**	**Contribution (%)**	**Trait category**	**Relation**	**Contribution (%)**
Animal-piercer	<	15.75	0.5-1cm	>	11.55	Deposit-feeder	>	12.33
0.25-0.5cm	<	9.66	Scraper	>	10.18	Herbivorous-piercer	<	11.77
Deposit-feeder	>	8.96	2-4cm	>	9.28	0.5-1cm	>	10.59
Herbivorous-piercer	<	7.99	Herbivorous-piercer	<	9.10	4-8cm	<	10.35
Scraper	>	6.78	Filter-feeder	>	8.11	2-4cm	>	9.03
Predator	>	6.64	0.25-0.5cm	>	7.60	Filter-feeder	>	8.12
4-8cm	<	6.55	Deposit-feeder	>	6.89	Scraper	>	7.59
≤1year	>	5.97	4-8cm	<	6.71	Animal-piercer	<	6.26
>1year	<	5.73	Animal-piercer	<	6.31	1-2cm	<	6.12
0.5-1cm	>	5.67	Predator	>	6.04	Predator	>	4.86
1-2cm	>	5.60	1-2cm	>	5.15	0.25-0.5cm	<	4.34
2-4cm	>	5.34	≤1year	>	4.73			

weak shift from *r* to *K* strategies detected for poorly dispersing organisms can be attributed to the observed increase in the organisms' affinity for larger body sizes, longer life cycles and higher trophic positions (i.e., predators) along the successional gradient (i.e., the PACs). The tendencies detected for body size and life cycle duration were in accordance with recent studies: De Bie et al. (2012) indicated that poorly dispersing organisms of larger sizes need more time to arrive at new locations, and Ruhí et al. (2012) detected an increase in the number of individuals with longer life spans and later maturations over time. Furthermore, the increases in predation affinity are in agreement with the general tendency found in other studies concerning macroinvertebrate succession in aquatic ecosystems (Wiggins et al., 1980; Schneider & Frost, 1996; Wellborn et al., 1996; Ruhí et al., 2012). In poorly dispersing organisms the high predation affinity found in oldest ponds may be related to larger body sizes because large body sizes are an advantage for caching prey (Woodward & Hildrew, 2002; Brown et al., 2004; Harrison & Dobson, 2008). The predation affinity was also higher in oldest ponds, but, in this case, were not related to large body sizes. To the easily dispersing organisms, higher affinity for larger body sizes were found in youngest ponds because to active dispersers larger sizes maximize the dispersal distance (Jenkins et al., 2007). Rather, the increase in the affinity for predation with pond age in easily dispersing organisms may be closely related to the increase in prey abundance (Nicola et al., 2010).

Deterministic and historical contingent forces

Environmental characteristics are one of the first filters for macroinvertebrate configuration because only the organisms with functional characteristics that are adapted to live under the ecosystem's specific conditions are able to colonize it and establish themselves (Poff, 1997; Weiher et al., 1998; Belyea & Lancaster, 1999; Statzner et al., 2004; Bêche et al., 2006; Mellado-Díaz et al., 2008). Thus, because the manmade ponds were constructed under similar conditions, we expected that the macroinvertebrate communities functionally converge if deterministic forces drove the communities (Samuels & Drake, 1997; Fukami & Wardle, 2005). In contrast to our expectations, no functional convergence occurred for either the easily or the poorly dispersing organisms. Nevertheless, our results suggested that deterministic forces may have a greater influence on poorly dispersing organisms because they maintained very high similarity values in functional characteristics beginning with the first PAC (Fig. 9A). On the other hand, according to Drake (1991 and Chase (2003), our study highlighted the fact that historical contingent forces significantly contributed to taxonomic composition because poorly dispersing organisms diverge despite maintain very high functional similarity values over time (Fig 8A and 9A).

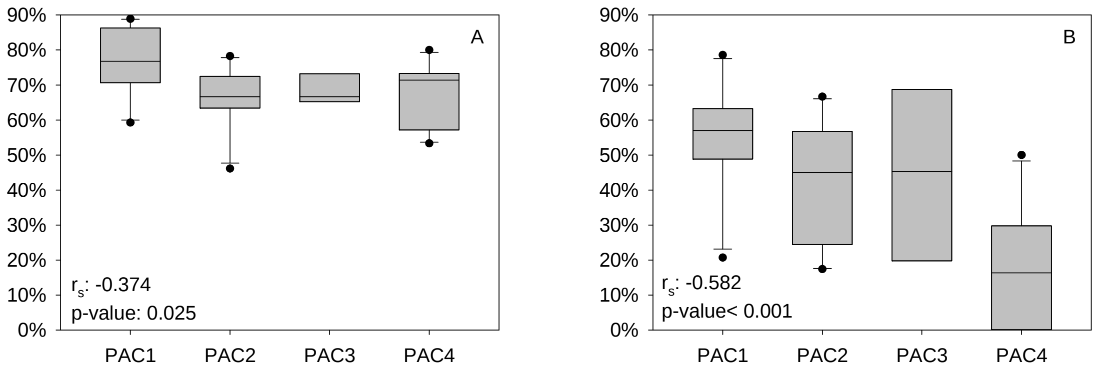

Figure 8: Variability of taxonomic similarity within each pond age category (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: >16 years). A: Data for poorly dispersing organisms; B: data for easily dispersing organisms. Spearman correlation for similarity among PACs is shown.

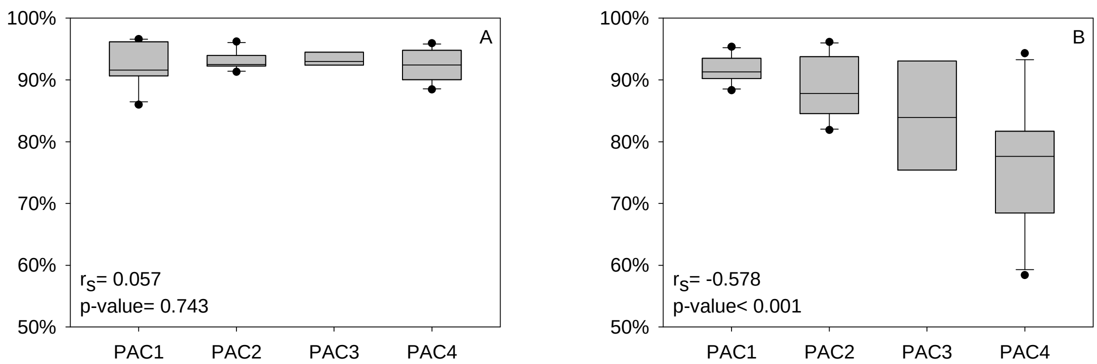

Figure 9: Variability of functional similarity within each pond age category (PAC; 1: 0-5 years, 2: 6-10 years, 3: 11-15 years, 4: >16 years). A: Data for poorly dispersing organisms; B: data for easily dispersing organisms. Spearman correlation for similarity among PACs is shown.

The differences found between poorly and easily dispersing organisms in their responses to deterministic or historical contingent forces may be based on their different life-history strategies (Wiggins et al., 1980; Harrison & Dobson, 2008; Verberk et al., 2008; Florencio et al., 2010). Because poorly dispersing organisms have a smaller chance of colonizing favorable new habitats, they most likely allocate higher resources to remaining more competitive and persisting in a place once they arrive, for example, through the production of large numbers of eggs and brood care (Verberk et al., 2008). In contrast, the easily dispersing organisms focus their strategy on colonizing new locations, which necessitates costly metabolic adaptations that reduce the resources that can be allocated to other functions, such as fecundity (Roff, 1990; Saglam et al., 2008). Therefore, stochastic drift could play a greater role in the configuration of easily dispersing rather than poorly dispersing organisms because their greater mobility assumes a higher risk of failure while locating a suitable new site (Masters et al., 2007), such as suffering predation *en route* (Malmqvist, 2002) and failing to locate mates (Harrison & Dobson, 2008).

CONCLUSIONS

This study about primary succession in lentic aquatic ecosystems was performed in a unique place, using ponds constructed at reclaimed opencast coal mines over the span of 22 years old. Under these conditions, we did not detect a clear evolutionary trend in the macroinvertebrate community, and we detected a weak shift from *r* to *K* strategies only for poorly dispersing organisms. Moreover, our results showed that historical contingent forces may have stronger influence than the deterministic forces in the final configuration of macroinvertebrate communities. Additionally, we found that easily dispersing organisms were more sensitive to stochastic drift and biotic interactions than were poorly dispersing organisms. Our study offers two interesting contributions: on the one hand, as we suggested, macroinvertebrates showed different responses during primary succession depending on their dispersal abilities; on the other hand, suggest that isolated ponds at reclaimed opencast coal mines are a limiting environment for the development of macroinvertebrate communities.

GENERAL DISCUSSION

Pond evolving: environmental changes and primary succession

The selected 19 manmade ponds, although covering a range from 1 to 22 years old, only showed weak differences related to pond age in both the environmental characteristics and the macroinvertebrate assemblages.

Limited information is available about the physical and chemical changes that occur in ponds with different successional stages (Angelibert et al., 2004; Magnusson & Williams, 2006). Physicochemical comparisons are rarely used to identify environmental trends among ponds of different ages (but see Hart & Davis, 2011; Ruhí et al., 2012). Rather, studies usually focused on biological dynamics and associated environmental factors (Takamura et al., 2008; Becerra Jurado et al., 2009), or were performed in temporary ponds where hydroperiod plays a very important role (Boix et al., 2004; Magnusson & Williams, 2006). The Chapter 1 showed no tendencies in water characteristics of the manmade ponds during time, only Fe slightly decrease with pond age. Meanwhile, in sediment, conductivity, total sulfur, Al, As, Fe and Zn increased with pond age. Moreover, in Chapter 2 a clear decrease in dissolved nitrogen after the first PAC and a progressively increase of total organic carbon, nitrogen and phosphorus in the sediment among PACs has been shown. In addition, a multivariate approach of environmental characteristics of the manmade ponds revealed that youngest ponds (PAC1; 1-5 years old) were statistically different in water and sediment characteristics than the ponds belonging to the PAC 2 to 4 (Chapter 2). These results suggested that, although the pond characteristics progressively change during time, the differences among the youngest ponds and the rest of the manmade ponds were greater. The decrease of dissolved nitrogen after the first years of pond construction is characteristic of coal mining effluents and have its origin in the nitrogen contained in coal (1-2%) that was rapidly leached from spoil mines (Pommen, 1983; Tiwary, 2001; Mishra et al., 2008). The use of explosives is another source of nitrogen in coal mines (Pommen, 1983; Griffith et al., 2012), but blasting was rare in the study area. The increase of total organic carbon, nitrogen and phosphorus in the sediment suggested an increase of the organic matter during time. This accumulation of organic matter may be mainly due to the accumulation of *Typha* sp. that have annual life cycle (Angelibert et al., 2004; Anderson & Mitsch, 2006; Hart & Davis, 2011). The increase of sulfur and total metals in the sediment evidenced a continuous arrival of these elements into the ponds. This incoming of sulfur and metals were probably related to the runoff produced during storm events resulting from the dissolution of metals and the direct erosion of the overburden materials. But the similarity in environmental characteristics among PAC2 to 4 (Fig 6B, Chapter 2) suggested that the release of metals and the erosion into the pond basins were progressive slowing down with time. This reduction in the metal incoming may be related to the progressive covering of the pond basins because vegetation increases the infiltration and reduces the runoff and the erosion probabilities (Moreno-de las Heras et al., 2008, 2009). In addition, it is important to highlight that water characteristics such as the pH, conductivity, alkalinity and concentration of SO_4^{2-} and dissolved heavy metals in the water, and the concentration of extractable metals in the sediment did not change over time. These results suggested that manmade ponds was able to maintain constant levels of the most bioavailable metal forms over time by its stabilization in the sediment and thus reducing the negative metal effects over the flora and the fauna (Sheoran & Sheoran, 2006; Merricks et al., 2007). This process was probably favored by the carbonate minerals found in the limestone substrate of this region (Pond et al., 2008; Bernhardt & Palmer, 2011; Griffith et al., 2012).

The study of the aquatic invertebrates inhabiting the manmade ponds showed few changes in the structure and the composition of the community over time (Chapters 2 and 3). Nevertheless, the analysis of these changes contributed to explain relevant aspects about the primary succession. Our findings reinforce the idea that the study of biodiversity over time requires the consideration of complementary aspects of biodiversity and not only the use of taxonomic richness. As a measure of biodiversity, taxonomic richness captures only some aspects of the composition of ecological assemblages and it does not account for phylogenetic, taxonomic, and functional variability among species in a community (Heino et al., 2005; Abellán et al., 2006). The use of complementary biodiversity metrics may provide valuable information about the status of ecosystems and may help to better understanding of community configuration over time (Collinson et al., 1995; Clarke & Warwick, 1998; Heino et al., 2005; Gascón et al., 2009). Moreover, the structure and the complexity of biotic assemblages could change over time although the number of taxa maintains constant (Bossuyt et al., 2003; Spieles et al., 2006; Hart & Davis, 2011). Therefore, the quantification of complementary biodiversity metrics, as the 'relatedness' of species within ecological assemblages (taxonomic distinctness) and the rarity is strongly recommended in combination to the study of taxonomic richness (Warwick & Clarke, 1995; Wilsey et al., 2005; Heino et al., 2007; Gascón et al., 2009).

In addition, our results revealed that, in isolated ponds, the evolving of the macroinvertebrate community greatly depends on the dispersal abilities of the organisms (Chapter 3). Dispersal is an important constraint in the changes of the communities related to the specific dispersal abilities of the pool of potential colonists, the proximity among localities within a region, and other landscape attributes as barriers and ecological corridors (Belyea & Lancaster, 1999; Chase, 2003). Therefore, differences in the evolving of poorly and easily dispersing organisms from the same pool of colonist was expected during primary succession in isolated manmade ponds. Accordingly, our study showed that organisms with poorly dispersing abilities had higher similarity in the taxonomic and functional composition than easily dispersing organisms over time (Chapter 3). Moreover, during primary succession, poorly dispersing organisms followed a shift from *r* to *K* strategy as was traditionally proposed (MacArthur & Wilson, 1967; Townsend & Hildrew, 1994), while easily dispersing organisms change in a less predictable way (Chapter 3).

Pond age was not the main important factor explaining the community changes. Water, sediment and landscape characteristics of the manmade ponds explained a greater proportion of macroinvertebrate biodiversity than pond age. Pond age encompasses the changes in the community due to variations in the environmental conditions and the community dynamics produced during the maturation of the manmade ponds. The functional differences found among youngest ponds (PAC1) and the other three PAC (Table 6, Chapter 2) coincide with the environmental differences found in water and sediment (Fig. 6 A and B). So, the environmental conditions may be explaining the functional changes among PAC (Belyea & Lancaster, 1999; Statzner et al., 2004; Griswold et al., 2008). In addition, the absence of taxonomic changes over time, the weak changes in the macroinvertebrate biodiversity and the high values of functional similarity among and within pond age categories (Chapters 2 and 3), suggested that environmental conditions may be limiting the community evolving and strongly determining the macroinvertebrate composition (Poff, 1997; Belyea & Lancaster, 1999; Statzner et al., 2004). On the other hand, progressively increase of biodiversity with pond age (Fig. 5, Chapter 2) did not coincide with significant environmental changes. Moreover, the study of the similarity among ponds within the same PAC (Figures 8 and 9, Chapter 3) revealed that, despite the maintenance of high functional similarity among ponds of the same PAC, the poorly dispersing organisms diverged in taxonomic compositions. So, these taxonomic changes may be explained by stochastic drift and biotic interactions (Drake, 1991; Chase, 2003; Lepori & Malmqvist, 2009). Therefore, our study suggests that both deterministic and historically contingent forces (stochastic drift and biotic interactions) were involved in community assemblage (Belyea & Lancaster, 1999; Chase, 2007; Lepori & Malmqvist, 2009) but with different effects over taxonomic and functional levels (Samuels & Drake, 1997; Fukami & Wardle, 2005).

The changes detected during primary succession of the macroinvertebrate community (Chapters 2 and 3) were not associated to successional phases. The observed changes were weak and we did not found typical taxa and functional trait categories associated to each PAC. In other works conducted in temporary (Lake et al., 1989; Bazzanti, 1996; Boix et al., 2004) and permanent wetlands (Ruhí et al., 2012), the changes in the community were related to three successional phases although differ in the nature of the third one. The first successional phase is allogenic; the second one is autogenic; and the third one is allogenic in a temporary pond due the desiccation of the pond, and autogenic in permanent ponds because of the environmental conditions did not significantly change. The absence of sequential changes during primary succession of manmade ponds was also found in Jeffries (2010) and Marchetti et al. (2010). Jeffries (2010) suggested that historical contingent forces played an important role in the absence of successional phases. Nevertheless, we should also consider the possibility that our study design was masking some changes of the macroinvertebrate evolving. The use of chronosequence approaches are generalized in macroinvertebrates and plants studies (e.g., Bossuyt et al., 2003; Marchetti et al., 2010; Hart & Davis, 2011; Helsen et al., 2012), but may increase the possibilities of taxonomic composition homogenization due to the dispersion of organisms among sites (Botts, 1997; Majer & Nichols, 1998). Even so, this seems not to be our case because we found that taxonomic composition among and within PAC diverged in its similarity over time.

Despite the absence of clear successional phases, it is possible that the macroinvertebrate community in the studied manmade ponds followed similar successional patterns than those underlined in Ruhí et al. (2012), where allogenic forces had a greater importance during first years after pond construction and then autogenic forces increased in importance. We were unable to test what happened during the first stages of macroinvertebrate succession due to the resolution of our work (pond data from 1 to 5 years old were grouped in the same age category). But we have found slightly differences in the functional composition of the PAC1 in comparison to the other PACs (Chapter 3). Moreover, we detected that during the first years after mining reclamation (PAC1), the environmental characteristics of the manmade ponds were different to the other PACs (Chapter 2). So it is possible that allogenic forces had a greater importance during the first years of the community evolving. In addition, the biodiversity increased when environmental conditions become more stable (PAC 2 to 4; Chapter 2), which suggested that autogenic forces may be more relevant in older ponds.

Reclaimed opencast coal mines: a limiting environment to pond evolving

The isolated manmade ponds constructed in the reclaimed opencast coal mines are a limiting environment to the evolving of the aquatic community. We have noted that the macroinvertebrate community showed low biodiversity values and reduced changes in their composition over time (Chapters 2 and 3). Moreover we found that pond age was not the main factor explaining the variability of pond biodiversity (Chapter 2). All this suggested the existence of environmental constrains to the development of the macroinvertebrate community in our study site.

The organisms should be functionally adapted to the ecosystem characteristics to colonize and established (Poff, 1997; Weiher et al., 1998; Belyea & Lancaster, 1999; Statzner et al., 2004; Bêche et al., 2006; Mellado-Díaz et al., 2008). Moreover, it is known that macroinvertebrates respond predictably to changes in local environmental conditions (Griswold et al., 2008). Therefore, the low environmental differences among ponds of PACs 2 to 4 (Chapter 2) could be responsible of the weak community changes. However, Ruhí et al. (2012) found important community changes (three successional phases) when environmental variables remain stable, so other environmental factors may be explaining the weak community evolving. We detected low habitat heterogeneity in the manmade ponds: the littoral of the ponds were dominated by *Typha* sp. and the ponds had similar sediment granulometry. This fact may reduce the macroinvertebrate biodiversity because faunal biodiversity is positively correlated to the complexity of habitat heterogeneity (O'Connor, 1991; Pedruski & Arnott, 2011). Moreover, we detected heavy metals in toxic concentration to the aquatic organism in the water and the sediment. This metal pollution may restrict the taxa able to settle in the ponds producing a loss of sensitive taxa and a shift in community composition toward more tolerant taxa (Clements, 1994; Gerhardt et al., 2004; Van Damme et al., 2008; Iwasaki et al., 2009). The recruitment of organisms may be a particularly important limitation to the development of the macroinvertebrate community in our study site. The manmade ponds were isolated and we did not found natural wetlands near the reclaimed mines, which mainly restrict the pool of potential colonist to the nearest streams. Lotic and lentic macroinvertebrates usually have different assemblage compositions (Williams et al., 2004; Biggs et al., 2007), and lotic taxa are considered poor dispersers compared with lentic taxa (Ribera & Vogler, 2000; Marten et al., 2006). Thus, dispersal constrains may be reducing the biodiversity and limiting the primary succession in the manmade ponds (Belyea & Lancaster, 1999; Brady et al., 2002; Brederveld et al., 2011). Indeed, smaller differences in community composition with increasing pond age where found when ecosystems were more isolated (Bossuyt et al., 2003).

Evaluation and Management recommendations to mine reclamation

From the perspective of the health of the aquatic ecosystems, mine reclamations fail in the control of metal pollution because we have detected heavy metals in concentrations that could be toxic to the aquatic organisms in the manmade ponds (MacDonald et al., 2000; US EPA, 2002b; Iwasaki et al., 2009). The manmade ponds were mainly isolated from water courses, which may protect downstream ecosystems from mine pollution. But there is the possibility that metal pollution affects downstream ecosystems during high storm events (Mitsch & Gosselink, 2000; Griffith et al., 2012). In these moments, the runoff could mobilize the metals accumulated in the sediment of the manmade ponds, increasing the load of metals in the water that come out on the reclaimed mines. In our study area, this may only happen in some manmade ponds, where, although the pond basin was endorheic, if water surface overcome a determined level, the manmade pond connect with the natural ecosystems. Additionally the effects of metal pollution could have negative effects outside the manmade ponds through the food web by bioaccumulation (Braune et al., 1999; Parker, 2004; Croteau et al., 2005). Even so, reclamation of opencast coal mines has been proven essential to develop functional aquatic ecosystems. Without mine reclamations, it is probably that pit-lakes with acidic pH, high polluted concentrations of heavy metals and absence of vegetation and aquatic organisms were formed, similar to the sampled pit-lake located in the un-reclaimed mine (Chapter 1).

The first objective in mine reclamations is to eliminate or reduce the negative effects outside of the mining areas. With this in mind, any action that improves the quality of reclaimed lands is going to contribute to reduce offsite mining effects. We found that one important environmental problem in the manmade ponds derived of coal mining was the metal pollution. Toxic concentrations of metal to the aquatic organisms remains 22 years after the mine reclamation finished (Chapter 1). In this study, we did not investigate about the sources and the forms in which metals income to the manmade ponds. Therefore, we could not propose specific actions to reduce metal pollution. But we suggested that most of metal pollution arrives through the runoff generated in the storms (Chapter 1). Indeed, in some reclaimed areas, the erosion uncovered subsurface layers of the overburden which showed coal content (see pictures 20 to 23 from appendix 1). Therefore, in order to reduce metal pollution the first step could be to reduce the soil erosion. Thus, the combined consideration of slope construction (with soft slopes), topsoil covering (including the base of the ponds) and plant establishment (by using the appropriate seed selection and limiting grazing) is fundamental to reduce sediment yield and erosion in reclaimed mining areas (Nicolau, 2003; Moreno-de las Heras et al., 2009; Martín-Moreno et al., 2013).

On the other hand, when manmade ponds are expected to stay integrated in the landscape without use restrictions (as is our study case), reclamation plans should include the improvement of other functions whenever possible and not produces interference with the runoff management. In Chapter 2 we showed that the manmade ponds had low values of macroinvertebrate biodiversity. If the metal pollution is reduced, it is possible that biodiversity in the manmade ponds increases. Nevertheless, to reduce the metal concentrations in the manmade ponds may be a very difficult issue. One easier action to improve the macroinvertebrate biodiversity may be increase the habitat complexity. Habitat structure is one of the fundamental factors determining the distribution of organisms at all spatial scales (O'Connor, 1991; McAbendroth et al., 2005; Pedruski & Arnott, 2011). Thus, the construction of softer bank slopes could increase the littoral area able to be colonized by plants and animals which could increase the biodiversity of the manmade ponds (Gee et al., 1997; Butler & de Maynadier, 2007; Markwell & Fellows, 2008). Moreover, the improving of particle size variability in the sediment of the manmade ponds (that rarely overcome the 2 mm) by the introduction of bigger particles, as gravel and cobble, could contribute to the increase of biodiversity of organisms (Beisel et al., 2000; Boyero, 2003). The littorals of these ponds were mainly covered by *Typha* sp. Different information exists about the effect of plant diversity in macroinvertebrate community. Several studies found positive influence of plant diversity and density in macroinvertebrate communities (Olson et al., 1995; Berg et al., 1997; Céréghino et al., 2008b; Williams et al., 2008); while in other cases, vegetation diversity did not improve the macroinvertebrate diversity (McAbendroth et al., 2005; Spieles & Horn, 2009). Therefore, improving the plant biodiversity in the manmade ponds through the introduction of several types of emergent macrophytes and submerged plants should be considered in the pond design because no negative effects over the macroinvertebrate community have been found. Macrophytes significantly contribute to the retention of metals in the sediment due to their effects on hydrology, sediment chemistry and microbial activity (Dunbabin & Bowmer, 1992; Mitsch & Wise, 1998; Ye et al., 2001; Marchand et al., 2010). For this reason, it is important to maintain a wide area of macrophytes in the shore of the manmade ponds. But the maintenance of a good coverture of littoral macrophytes may be combined with designing little areas without vegetation to contribute to the diversity because the community composition and abundance of macroinvertebrates from vegetated and un-vegetated areas are different (Olson et al., 1995; Della Bella et al., 2005). In addition, the construction of intermediate isolated ponds among the streams and the mine areas may increase the biodiversity by improving the connectivity among aquatic ecosystems (Oertli et al., 2008; Williams et al., 2008; Martínez-Sanz et al., 2012).

Mine reclamation could be an opportunity to experiment about the ways of improving metal retention and increasing the biodiversity because there is the possibility of constructing replications to test different objectives. These objectives should be included in mine reclamation plans before mine exploitation begins and the results obtained could be used to improve the quality of mine reclamations in future projects through adaptive management (Pastorok et al., 1997; Cummings et al., 2005; Reever Morghan et al., 2006).

Manmade pond services

The manmade ponds were designed to control the runoff generated within the reclaimed area but, despite metal toxicity, the manmade ponds may provide additional services (Ghermandi et al., 2010) as for example: habitat/refugia, biodiversity support, carbon management, gas regulation, disturbance regulation, water regulation, water supply, waste treatment, food production, raw materials or recreational activities (Costanza et al., 1997; Zedler & Kercher, 2005; Carpenter et al., 2011). Manmade aquatic ecosystems usually have a significant contribution to freshwater biodiversity even if they were not designed for biodiversity benefits (Hansson et al., 2005; Céréghino et al., 2008a; Thiere et al., 2009). Our results showed low macroinvertebrate biodiversity in the manmade ponds in the Teruel coalfield in comparison with other manmade ponds of similar ages (Chapter 2). In spite of this, the manmade ponds provide both taxonomic and landscape biodiversity, at least at regional scale, because there are no natural ponds in the study area. Moreover, other organisms as fish, amphibian, waterfowl and mammals are beneficed of pond construction, because the manmade ponds provide water, food and refuge (Mitsch & Gosselink, 2000; Scheffer et al., 2006). In addition, small aquatic ecosystems play a major role in ecological cycles such as global carbon cycle (Mitra et al., 2005; Zedler & Kercher, 2005; Downing, 2010). Rates of organic carbon sequestration in the sediments of ponds and small lakes has been suggested to be orders-of-magnitude greater than big lakes (Dean & Gorham, 1998; Downing et al., 2008; Downing, 2010). In our case, we have detected that carbon concentration in the sediment of manmade ponds increase with pond age (from 1 to 6%; Chapter 1) contributing to carbon sequestration. On the other hand, the manmade ponds in the study area may be used by the human population. As far as we know, people did not usually use these areas to recreation. Endesa, one of the companies that extracted coal in the study area, and local municipalities organize excursions to explain mine restoration and the characteristics of the constructed ponds which have educational and recreational values. Moreover, some of the oldest ponds are used for fishing (fishes were human introduced for fishing purposes) and to water sheep. Both activities may suppose a risk to human health since we know that metal pollution exists. Finally, since we were conducting our research in these manmade ponds they are also achieving a research function. Newly created habitats have proven to be ideal systems to test temporal community dynamics (Noon, 1996; Chase, 2007; Hassall et al., 2012; Ruhí et al., 2012) and may be of especial relevance in the study of primary succession because they are created on surfaces where an aquatic community has not previously existed and the age is usually known (Velasco et al., 1993; Flory & Milner, 2000; Matthews et al., 2009).

CONCLUSIONS

ENGLISH VERSION

Physicochemical differences among manmade ponds of different ages

- The physicochemical characteristics of the manmade ponds constructed for runoff control in reclaimed opencast coal mines showed weak although significant changes over time.
- In the water, only dissolved nitrogen and iron decrease over time; in the sediment conductivity, organic matter , total sulfur, aluminum, arsenic, iron and zinc showed an increasing trend.
- The youngest ponds (< 6 years after pond construction) were different in water and sediment characteristics than ponds from 6 to 22 years old.
- Differences between youngest and the rest of the manmade ponds were related to mine pollution: the water of youngest ponds showed higher dissolved nitrogen of mine origin and the older ponds showed higher metal concentrations in the sediment due to its accumulation.

Primary succession of macroinvertebrate community

- The macroinvertebrate community did not show significant differences in taxonomic composition over time. Moreover, the number of taxa maintains without significant differences along the studied period.
- The macroinvertebrate community increased in biodiversity during primary succession: the community increased in the phylogenetic distant among taxa (increase of AvTD), showed greater unevenness in the taxonomic tree (increase of VarTD), and increase in rarity (increase of IFO) with pond age.
- It is important to use complementary biodiversity metrics to understand the evolving of a community through time. If only taxonomic richness is employed, other changes in biodiversity of the assemblages will be overlooked.

- The study of the biodiversity revealed that age was not the main factor explaining the differences in community structure in the studied manmade ponds.
- Macroinvertebrate community responded simultaneously to many factors but environmental characteristics stand out over pond age.
- We did not detect successional phases during primary succession despite observe several changes in biodiversity and functional composition.
- Aquatic macroinvertebrates showed different responses during primary succession depending on their dispersal abilities.
- Weak although significant differences among pond age categories were found related to trait composition: whereas poorly dispersing organisms showed a shift from *r* to *K* strategies, easily dispersing organisms showed less predictable changes.
- The macroinvertebrate community showed high similarity over time, greater for the poorly than for the easily dispersing organisms.
- Both, deterministic and historical contingent forces (stochasticity drift and biotic interactions) revealed important in the community configuration. Poorly dispersing organisms showed greater influence from environmental characteristics while easily dispersing organisms showed a greater influence from historical contingent forces.
- Weak changes of macroinvertebrate assemblages over time suggest that the environmental conditions of this study area were restricting the evolving of the community.

Effectiveness of opencast coal mine reclamations

- The biodiversity of the manmade ponds constructed in reclaimed coal mines were lower than wetlands and ponds of similar ages constructed for other purposes in other environmental conditions.
- Dissolved Al, Cd, Cu and Ni in the water and total As and Ni in the sediment were found in toxic concentration to the aquatic organisms in the manmade ponds two

decades after mine reclamation. This suggests that metal pollution in the reclaimed coal mines of this area may be a chronic problem and one of the causes of the observed low biodiversity values.

- The comparison of the manmade ponds with the pit-lake revealed that reclamation of opencast coal mines is essential. In this area, if opencast coal mines were not reclaimed, there are high probabilities that acidic lakes with high toxic concentrations of heavy metals and unable to sustain aquatic life were formed.
- From an offsite effect point of view, reclamations are effective in the control of mining pollution if all the runoff generated inside mining area is directed towards endorheic manmade ponds. However, metal exportation to downstream ecosystems could be produced during high storm events and through the food web.
- Because the level of complexity of the community increases over time and natural ponds were not found in our study area, the studied manmade ponds are providing both biological and landscape diversity in the studied region.

SPANISH VERSION

Diferencias en la fisicoquímica de las balsas artificiales en función de su edad

- Las balsas artificiales construidas para retener la escorrentía en las minas de carbón a cielo abierto recuperadas muestran ligeros pero significativos cambios en sus características fisicoquímicas a lo largo del tiempo.
- En el agua, nitrógeno y hierro disminuyen con el tiempo; en el sedimento, la conductividad, materia orgánica y las concentraciones totales de azufre, aluminio, arsénico, hierro y zinc muestran una tendencia positiva con la edad.
- Las balsas más jóvenes (aquellas que tienen menos de 6 años de antigüedad) mostraron características en el agua y el sedimento distintas de las balsas entre 6 y 22 años de antigüedad.
- Las diferencias encontradas entre las balsas más jóvenes y las otras tres clases de edad estuvieron relacionadas con contaminación de origen minero: en el agua, las elevadas concentraciones nitrógeno disuelto probablemente proceden de la liberación del nitrógeno presente en el minera de carbón, mientras que en el sedimento de las balsas más antiguas la concentración de metales es mayor debido a su acumulación a lo largo del tiempo.

Sucesión primaria de la comunidad de macroinvertebrados

- La comunidad de macroinvertebrados no mostró diferencias significativas en su composición taxonómica a lo largo del tiempo. Además, no se encontraron diferencias en el número de taxones por balsa entre las distintas clases de edad.
- La biodiversidad de la comunidad de macroinvertebrados creció durante la sucesión primaria: la comunidad incrementó la distancia filogenética entre taxones (aumento de la AvTD), mostró una mayor heterogeneidad en el árbol taxonómico (aumento de la VarTD) y aumentó en el número de taxones raros (aumento del IFO) con la PAC.

- Es importante considerar aspectos complementarios de la biodiversidad de la comunidad para entender su evolución a lo largo del tiempo. Si sólo se considera la riqueza de taxones, otros cambios en la diversidad de la comunidad serán omitidos.
- El estudio de la biodiversidad mostró que la edad no fue el factor que mejor explica las diferencias en la estructura de la comunidad en las balsas artificiales.
- La comunidad de macroinvertebrados respondió simultáneamente a varios factores, aunque destacaron las características ambientales sobre la edad de las balsas.
- No se detectaron fases diferenciadas durante la sucesión primaria a pesar de haber encontrado algunos cambios en la biodiversidad y en la composición funcional de la comunidad a lo largo del tiempo.
- Los macroinvertebrados acuáticos mostraron diferentes respuestas en función de su capacidad de dispersión a lo largo de la sucesión primaria.
- Ligeras aunque significativas diferencias entre balsas de distintas clases de edad fueron encontradas en relación a la composición funcional: mientras que los malos dispersores mostraron un cambio desde la estrategia *r* hacia la *K*, los buenos dispersores mostraron cambios menos predecibles.
- La comunidad de macroinvertebrados mostró una elevada similaridad funcional a lo largo del tiempo, aunque mayor en los malos que en los buenos dispersores.
- Tanto las fuerzas deterministas como las históricas contingentes (deriva estocástica y relaciones entre los organismos) fueron importantes en la configuración final de la comunidad de macroinvertebrados. Los malos dispersores mostraron una mayor respuesta a los cambios ambientales mientras que los buenos dispersores mostraron una mayor respuesta a las fuerzas históricas contingentes.
- Que los macroinvertebrados no muestren importantes cambios a lo largo del tiempo sugiere que las condiciones ambientales de esta zona podrían estar restringiendo el desarrollo de la comunidad.

Efectividad de la recuperación de las minas de carbón a cielo abierto

- La biodiversidad de las balsas artificiales fue menor que la biodiversidad encontrada en humedales y balsas de edad similar construidas en con otros fines en condiciones ambientales diferentes.
- Las concentraciones de Al, Cd, Cu y Ni en el agua y de As total y Ni total en el sedimento registraron valores tóxicos para los organismos acuáticos. Similares concentraciones se encontraron entre las balsas más jóvenes y aquellas con más de 20 años de edad. Esto sugiere que la contaminación por metales en las minas de carbón recuperadas podría ser un problema crónico y una de las causas de la baja biodiversidad detectada.
- La comparación de las balsas artificiales con el pit-lake mostró que la recuperación de minas de carbón a cielo abierto es un asunto esencial. En esta zona, si las minas de carbón a cielo abierto no se recuperan, existe una elevada probabilidad de que los lagos que se formen en ellas sean ácidos y con elevadas concentraciones de metales pesados sin posibilidades de albergar vida acuática.
- Desde el punto de vista de los efectos fuera de las minas, la recuperación de las minas sería efectiva siempre que la escorrentía generada sea dirigida hacia balsas endorreicas. Sin embargo, la exportación de metales a los ecosistemas naturales podría producirse durante fuertes tormentas y la cadena trófica.
- Debido a que el nivel de complejidad de la comunidad aumentó con el tiempo y a que no existen balsas o humedales en esta región, las balsas artificiales construidas para retener la escorrentía incrementan la biodiversidad biológica y paisajística de la región estudiada.

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APPENDIX 1: PICTURES

Sequence of mine closing and pond construction

Picture 1: Sequence of mine closing and pond construction in Corta Alloza, an opencast coal mine at northeast of Spain belonging to Endesa S.A. company (ponds number 17 and 18)

Studied manmade ponds

Picture 2: Manmade pond number 1

Picture 3: Manmade pond number 2

Picture 4: Manmade pond number 3

Picture 5: Manmade pond number 4

Picture 6: Manmade pond number 5

Picture 7: Manmade pond number 6

Picture 8: Manmade pond number 7

Picture 9: Manmade pond number 8

Picture 10: Manmade ponds number 9 (left) and 10 (right)


Picture 11: Manmade pond number 11


Picture 12: Manmade ponds number 12


Picture 13: Manmade pond number 13

Picture 14: Manmade pond number 14

Picture 15: Manmade pond number 15

Picture 16: Manmade pond number 16

Picture 17: Manmade ponds number 17 (left) and 18 (right)

Picture 18: Manmade pond number 19

Picture 19: Pit-lake of un-reclaimed mine,number 20

Erosion and overburden exposure in reclaimed mines

Picture 20: Erosion and overburden exposure

Picture 21: Erosion and overburden exposure

Picture 22: Erosion and overburden exposure

Picture 23: Erosion and overburden exposure

APPENDIX 2: FAUNAL LIST

Presence/absence faunal list organized by pond age categories (PAC)

		PAC			
GROUP	TAXA	1	2	3	4
Coleoptera	Donacia	■	■	■	
	Dryops	■			
	Acilius	■	■		■
	Bidessus	■	■		
	Coelambus	■	■		
	Copelatus		■		
	Eretes				■
	Graptodytes	■	■	■	■
	Hydroglyphus	■			
	Hydroporus	■			
	Hygrobia		■		
	Hygrotus		■		
	Hyphydrus	■	■	■	
	Ilybius	■	■	■	■
	Laccophilus	■	■	■	■
	Meladema				■
	Scarodytes				■
	Yola	■	■		■
	Limnius	■			
	Gyrinus		■		
	Haliplus	■	■	■	■
	Helophorus		■	■	
	Limnebius				■
	Berosus	■	■	■	■
	Hydrochara		■		
	Helochares	■	■	■	
	Laccobius				■
	Hydrochus			■	
	Noterus		■	■	■
	Hydrocyphon				■
Diptera	Ceratopogonidae	■	■	■	■
	Orthocladiinae	■	■	■	■
	Quironomini	■	■	■	■
	Tanipodinae	■	■	■	■
	Tanitarsini	■	■	■	■
	Anopheles	■	■	■	■
	Culex		■		■
	Culiseta				■
	Dixella		■	■	■
	Dolichopodidae	■	■		■
	Hydrellia	■			
	Scatella	■	■		■
	Dicranomyia		■	■	
	Helius		■	■	■
	Pilaria				■
	Rhypholophus	■			

Presence/absence faunal list organized by pond age categories (PAC) continuation

		PAC			
GROUP	TAXA	1	2	3	4
Diptera	Tr. Eriopterini				■
	Pericoma				■
	Tenatocera	■			
	Nemotelus				■
	Odontomyia		■		
	Oxycera		■		■
	Tipula				■
Ephemeroptera	Cloeon	■	■	■	■
	Procloeon			■	
	Caenis	■	■	■	■
	Ephemera	■	■	■	■
	Thraulus		■		■
	Corixa	■	■	■	■
	Cymatia	■	■	■	■
	Micronecta	■	■	■	■
	Sigara		■		
	Gerris	■	■	■	■
	Mesovelia			■	
	Naucoris	■	■	■	■
	Anisops	■		■	
	Noctonecta	■	■	■	■
	Plea	■	■	■	■
Megaloptera	Sialis				■
Odonata	Anax	■	■	■	■
	Aeshna		■		■
	Gomphus		■		■
	Onychogomphus		■		
	Coenagrion	■	■	■	■
	Ceriagrion		■		
	Ischnura	■	■	■	■
	Lestes	■	■	■	■
	Sympecma	■	■	■	■
	Crocothemis	■	■	■	
	Libellula			■	
	Orthetrum	■	■	■	■
	Sympectrum	■	■	■	■
	Platycnemis				■
Trichoptera	Ecnomus			■	■
	Allotrichia	■	■	■	
	Setodes	■		■	■
	Micropterna				■
	Agrypnia				■
	Plectrocnemia	■			
Oligochaeta	Tubificidae	■	■	■	■

Presence/absence faunal list organized by pond age categories (PAC) continuation

		PAC			
GROUP	TAXA	1	2	3	4
Bivalva	Pisidium				■
Gastropoda	Potamopyrgus				■
	Galba		■		
	Radix		■		■
	Physa	■	■	■	■